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Articles for publication in the Journal must bear the formal approval of the chief of the department bureau, or of the director of the experiment station from which the paper emanates. Each manuscript must be accompanied by a statement that it has been read and approved by one or more persons (named) familiar with the subject. The data as represented by tables, graphs, summaries, and conclusions must be approved from the statistical viewpoint by someone (named) competent to judge. All computations should be verified

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ERRATA AND AUTHORS' EMENDATIONS

Page 35, paragraph 3, line 5, *"timopheevizhuk"* should be *"timopheevi* Zhuk."

Page 175, table 1, column 4, *"s(t)"* in box head should be *"σ(t)."*

Plates 2 and 4 (facing pages 277 and 279), "see next page" should be "see preceding page."

Plate 5 (facing page 280), *"C,"* omitted from the explanatory legend, should be two lines below *"B."*

Page 343, table 2, last column, box head should read "Average yield per acre for the years used in statistical analysis," and *"Years"* below box should be *"Bushels."*

Page 346, in heading at top of page, "NO" should be "ON."

Page 455, paragraph 3, line 3, "nitrate" should be "nitrite."

Page 465, table 6, next to last compound, *"d"* should be Greek delta.

Page 494, figure 3, the range of milligrams per gram on the vertical scale should be .20 to 1.40 instead of 20 to 140.

Page 499, in the two tables starting on line 25 in which the localities under "Variety and locality" are transposed. Kennebunk should precede Aroostook and fall under A in 1938 and Aroostook should fall under A in 1939.

Page 510, legend for figure 2 belongs to figure 3, page 512, and vice versa.

Page 586, paragraph 3, line 8, "1 00" should be "1 100."

Page 603, footnote 2, "Average of values" should read "Average of first 4 values."

Page 622, structural formulas are wrong: (1) Carbon atoms not in line; (2) *D-*fructose should have "CO" in the "2" position instead of "HOCH."

Page 623, paragraph 1, last line, *"L-*sorbose" should be omitted.

Page 649, table 2, *"6-*Alanine" should be *"β-*Alanine."

Page 658, paragraph 1, last line, "table 3" should be "table 4."

Page 677, first formula, the "C" in right-hand phenyl group should be "C1," and should be attached to the right center instead of at bottom.

Page 687, paragraph 2, line 4, the last genotype in the line, "$S^1S\ Z^2Z^3$," should be "$S^1S^2Z^2Z^3$."

Page 691, third line from bottom, "and S^7Z^6 gametes" should be "and S^1Z^6 gametes."

REDUCING SOUTHERN SCLEROTIUM ROT OF SUGAR BEETS WITH NITROGENOUS FERTILIZERS [1]

By L. D. LEACH, *associate plant pathologist*, and A. E. DAVEY, *junior pomologist*, California Agricultural Experiment Station [2]

INTRODUCTION AND LITERATURE REVIEW

Several workers have advocated the use of soil amendments for controlling plant diseases caused by soil fungi. King and Loomis (8)[3] reported that heavy applications of manure or other organic material consistently reduced the number of cotton plants dying from phymatotrichum root rot. According to later work by King, Hope, and Eaton (9) and King (7), the benefits of organic manures resulted from inability of the root rot fungus to thrive in the presence of great numbers of bacteria, fungi, and other saprophytic organisms that are actively engaged in decomposing organic material. Neal and his coworkers (15), on the other hand, in 1933 demonstrated the toxicity of ammonia and certain ammonium salts to mycelium of *Phymatotrichum omnivorum* (Shear) Duggar and suggested that the effect of manure on the root rot may depend upon the early or ammoniacal decomposition of the manure. The following year Streets (18) reported experiments in which phymatotrichum root rot on deciduous fruit and nut trees was controlled with anhydrous ammonia or ammonium sulfate. In a later publication (19) he recommended either ammonium sulfate or ammonium phosphate for this disease on a number of perennial crops. The beneficial results were attributed primarily to the killing of the root rot fungus by the strong ammonium sulfate solution, secondarily to the stimulation of the plants by the large amount of quickly available nitrogenous fertilizers. Jordan and others (6) furnished further evidence of the effect of fertilizer treatments in reducing the loss of cotton from this fungus.

Applications of chicken manure, horse manure, or any of several other organic materials reduced the severity of take-all of wheat caused by *Ophiobolus graminis* Sacc. in the experiments of Fellows (4), who suggested the antagonistic effect of the natural soil microflora as the mechanism responsible for reducing the disease.

Walker and Musbach (21), after reviewing the results of earlier workers, presented additional evidence that root rot of canning peas caused by *Aphanomyces euteiches* Drechsler can be appreciably reduced by applications of mineral nitrogenous fertilizers. They suggest that the plants develop greater resistance to infection when supplied with quickly available nutrients. In a recent paper Smith and Walker (16) reported that reduction in aphanomyces root rot of peas was correlated with increase in total salt concentration of the nutrient

solution and was not the specific effect of nitrogen, phosphorus, or potassium salts.

Club root of cabbage has been partly controlled with nitrogenous fertilizers. Under certain conditions, according to Walker and Larson (*20*), field applications of 400 to 800 pounds of $CaCN_2$ were fairly effective; and this material appeared about twice as effective, pound for pound, as $Ca(OH)_2$.

Early results with *Sclerotium rolfsii* Sacc. were not encouraging for this line of attack. Edgerton and Tims (*3*), reporting trials with 26 fertilizer combinations during two seasons in Louisiana, found no clear-cut differences between the various treatments. Molz (*14*), on the other hand, working in the island of San Miguel (Azores) with a sugar-beet root rot which he attributed to *Typhula betae* Rostr., recommended suitable fertilizers because the disease appeared less severe in fields abundantly provided with artificial fertilizers.

Although the writers have had no opportunity to examine cultures of *Typhula betae*, Dr. E. W. Schmidt, Zuckerfabrik Klein-Wanzleben, Magdeburg, Germany, has kindly furnished two cultures of *Typhula variabilis*, about which he made the following comment: "I take the liberty of sending you * * * a culture of a fungus * * * known in Europe under the name *Typhula variabilis*, which is very rare in this country and which appears only in dry-warm climates, as for example now and then in Italy and in Spain. I have found this fungus on sugar beet in South Spain near Sevilla. The symptoms of disease are exactly the same as those of *Sclerotium rolfsii*. Also in cultural respects, the fungus behaves in the same way, so that it is to be presumed that *Typhula variabilis* and *Sclerotium rolfsii* are possibly identical.[4] "

Examination of these cultures and observations on their growth on sugar beets have convinced the writers that this fungus is indistinguishable from *Sclerotium rolfsii*.

Encouraged by the report of Neal, Wester, and Gunn (*15*) of the toxicity of ammonia to *Phymatotrichum omnivorum* and by the partial control of the root rot caused by that fungus by means of either organic or inorganic nitrogenous fertilizers (*7, 9, 18, 19*), the writers have conducted laboratory and field experiments to determine the possible application of similar methods for controlling *Sclerotium rolfsii* on sugar beets. The most striking feature of the results (*11, 12*) has been the consistent reduction of infection in all trials where nitrogenous fertilizers have been heavily applied. The observed results might be explained by any of the following hypotheses: (1) Direct toxicity of the chemicals or their decomposition products to the sclerotia or mycelium of *S. rolfsii;* (2) creation of a soil medium less favorable to the growth or parasitism of the casual fungus; (3) alteration in the physiology or anatomy of the host as the result of nitrogen absorption that might decrease the susceptibility of the host; (4) alteration in the balance of micro-organisms in the soil resulting in the suppression of *S. rolfsii* because of antagonism or competition.

The field and laboratory experiments described in the following pages show the efficacy of nitrogenous fertilizers in reducing losses of sugar beets from southern sclerotium rot and contribute some evidence toward the evaluation of these hypotheses.

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[4] Letter to Dr. J. B. Kendrick, dated February 19, 1935.

FIELD EXPERIMENTS ON THE EFFECT OF NITROGENOUS FER-
TILIZERS ON THE INCIDENCE OF *SCLEROTIUM ROLFSII* INFEC-
TION ON SUGAR BEETS

TRIALS ON SOILS OF LOW FERTILITY

In a field of sugar beets (*Beta vulgaris L.*) in Yolo County, Calif.,
with a uniform infestation of *Sclerotium rolfsii*, a series of plots was
established in the spring of 1934 to determine how heavy applications
of anhydrous ammonia or ammonium sulfate might affect the subse-
quent development of the disease. Ammonia, dissolved in the
irrigation water, was applied to six ⅛-acre plots at the rate of 142.5
pounds per acre (about 340 parts per million of irrigation water) on
June 1, and at 103.5 pounds per acre (about 240 parts per million)

FIGURE 1.- Effect of nitrogenous fertilizers on sugar beets in field of low fertility
but heavily infested with *Sclerotium rolfsii* a, Anhydrous ammonia 246 pounds
per acre, infection 13.5 percent, yield 17.1 tons per acre; b, nonfertilized,
infection 34.0 percent, yield 10 3 tons per acre. For the average results from
all plots see table 1. Similar results were obtained from ammonium sulfate
applications.

on June 15, 1934. In the same way ammonium sulfate was dissolved
in the irrigation water at the rate of 427.5 pounds per acre on each
of these dates and applied to six ⅛-acre plots. An equal number of
plots randomized among the fertilized plots were irrigated with
water alone.

Soon after the ammonia or ammonium sulfate was applied to the
plots the fertilized plants showed remarkable stimulation, as was
apparent from the height of foliage, the size of leaves (fig. 1), and a
change to a darker green in the color of the leaf. These responses
undoubtedly indicate that there had been a deficiency of available
nitrogen.

Counts of visibly infected beets in the middle four rows of each
plot were made at 10-day intervals, starting before the first applica-
tion was made and continuing until harvest on September 11. Mor-

tality curves for the fertilized and nonfertilized areas are presented in figure 2. On May 31, just before the first application, the average percentage of infection in the three groups of plots was approximately the same; but thereafter the mortality rate was much higher in nonfertilized plots than in those treated with either ammonia or ammonium sulfate.

All the plots were harvested on September 11 and 12, when final disease counts, yields, average root weights, and sucrose percentages were determined. Table 1 summarizes the results.
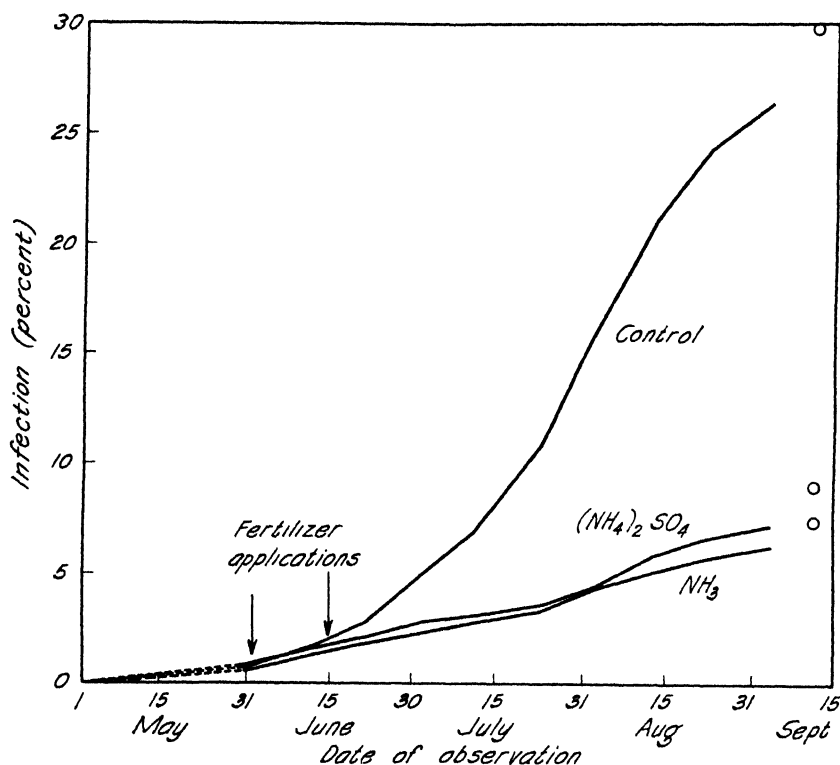
FIGURE 2. -Seasonal increase of *Sclerotium rolfsii* infection on sugar beets in fertilized and nonfertilized plots; average of six plots. The percentage of infection indicated by the circles over the date of September 12 includes the infected roots observed during harvest.

Infection was reduced from 30.0 percent in the nontreated plots to 7.6 and 9.2 percent in the plots treated with ammonia and ammonium sulfate. The nitrogenous fertilizers increased the average root weight by approximately 27 percent and depressed the sucrose percentage from 19.97 to 17.87 and 18.63 percent for ammonia and ammonium sulfate, respectively. The yield of sugar beets was increased from 11.5 tons to 19.9 and 19.6 tons per acre; the yield of gross sugar from 4,590 to 7,080 and 7,310 pounds per acre. To sum up, the percentage of disease-free plants, the average root weight, the yield of beets, and the gross sugar yield were significantly increased, while the sucrose percentage was significantly decreased, by both ammonia and ammonium sulfate.

TABLE 1.—*Comparative effects of anhydrous ammonia and ammonium sulfate applied in the irrigation water on the incidence of infection and the yield of sugar beets growing in soil of low fertility infested with Sclerotium rolfsii, 1934*

Treatment	Fertilizer applied per acre	Nitrogen applied per acre	Infected beets	Average root weight	Sucrose content of beets	Acre yield of—	
						Beets	Gross sugar [1]
	Pounds	*Pounds*	*Percent*	*Pounds*	*Percent*	*Tons*	*Pounds*
Control	None	None	30 0	1 20	19 97	11 5	4,590
Ammonia	2 246	200	7 6	1 51	17 87	19 9	7,080
Ammonium sulphate	3 855	176	9 2	1 53	18 63	19.6	7,310
Significant difference	{Odds 19 1		5. 69	. 20	81	2 60	805
	{Odds 99 1		8 10	28	1 16	3.70	1. 145
Calculated *F* value [4]			48 00**	8 54**	17 88**	33 21**	34. 76**

[1] Yield×sucrose percentage.
[2] Dissolved in irrigation water. First application, June 1, 142.5 pounds NH₃ per acre, second application, June 15, 103 5 pounds NH₃ per acre
[3] Dissolved in irrigation water at the rate of 427.5 pounds of ammonium sulphate per acre in each of 2 applications, June 1 and 15, 1934.
[4] **Exceeds the 1-percent point, F=7 56 (17).

Evidently the increase in yield of sugar in the plots treated with ammonia or ammonium sulfate resulted in part from reduction of infection and in part from stimulation of growth. The slight reduction in sucrose percentage in the fertilized plots was more than offset by the increase in root size and the number of surviving beets.

Inasmuch as *Sclerotium rolfsii* caused considerable loss in the plots treated in 1934 before the treatments were applied, it was thought that the nitrogenous fertilizers should be applied early in the season for the most effective results. The 1935 trials were made to determine whether broadcasting the dry forms of nitrogenous fertilizers would be an effective means of application and to compare the relative efficiency of three forms of nitrogenous fertilizers. The field in Sutter County selected for the trials was last planted to sugar beets in 1931, when a loss of about 25 percent from southern sclerotium rot occurred.

On December 24, 1934, applications of 100 or 200 pounds of nitrogen as ammonium sulfate, calcium nitrate, and Cyanamid were each distributed over the surface of five ½-acre plots, while an equal number of randomized plots were left untreated. The nitrogenous fertilizers were mixed with the soil by disking and harrowing, and the intention was to plant sugar beets after the lapse of 15 or 20 days. Because of heavy rainfall, however, planting was delayed until late in February; and then, because of unfavorable conditions, the stand was unsatisfactory. The field was replanted on April 1, and an excellent stand resulted. The beets grew satisfactorily until the second irrigation, when an excessive application of water to a poorly drained portion of the field resulted in the loss of numerous plants by "water rot" and necessitated the abandonment of one set (seven) of the experimental plots.

The remaining plots were harvested on September 4, 1935; the records appear in table 2. Although the percentage of infection is considerably less in the treated than in the nontreated plots, the differences between materials or rates of applications are not statistically significant. The explanation is, chiefly, that the number of replications was limited; that an area of severe infection occurred

in part of the two blocks; and that, despite chance randomization, some treatments were in a more favorable position than others.

TABLE 2.—*Comparative effects of various nitrogenous fertilizers, applied to the soil before planting, on the incidence of infection and the yield of sugar beets growing in soil of low fertility infested with Sclerotium rolfsii, 1935 and 1936*

1935 [1]

Treatment	Fertilizer applied per acre	Nitrogen applied per acre	Infected beets	Average root weight	Sucrose content of beets	Acre-yield of —	
						Beets	Gross sugar [2]
	Pounds	*Pounds*	*Percent*	*Pounds*	*Percent*	*Tons*	*Pounds*
Control	None	None	15 92	1 03	19 25	12 23	4, 700
Ammonium sulfate	485	100	7 82	1 19	19 17	16 53	6, 280
Calcium nitrate	645	100	5 32	1 23	19 54	18 04	7, 090
Cyanamid	475	100	8 35	1 16	19 18	15, 86	6, 080
Ammonium sulfate	970	200	6 45	1 37	19 02	18 36	6, 990
Calcium nitrate	1, 290	200	4. 12	1 43	18 11	19 79	7, 120
Cyanamid	950	200	6 87	1 28	18 08	17 69	6, 360
Significant difference	{Odds 19 1		7 31	. 099	1 17	3 23	1 260
	{Odds 99 1			136	1 60	4 42	1, 720
Calculated *F* value [3]			2. 44	15 38**	2 14	5 00**	5 37**

1936 [4]

Control	None	None	11 50	1 00	21 90	9 54	4, 160
Cyanamid	238	50	9 43	1 15	21 10	12 07	5, 110
Calcium nitrate	323	50	6 78	1 24	20 50	13 71	5, 600
Cyanamid	475	100	6 00	1 20	20 80	13 72	5, 680
Ammonium sulfate	485	100	7 36	1 20	20 30	13, 90	5, 650
Ammonium phosphate	623	100	9. 60	1 26	20 80	13 16	5, 440
Calcium nitrate	645	100	3 65	1 32	20 30	15 40	6, 250
Cyanamid	950	200	2 87	1 45	18 90	16 58	6, 250
Significant difference	{Odds 19 1		4 64	0 19	1 39	1 84	489
	{Odds 99 1		6 44	0 27	1 93	2 55	684
Calculated *F* value [5]			3. 79*	4 25*	3. 46*	12 11**	17 31**

[1] Date of planting, Apr. 1, 1935, date of application, Dec. 24, 1934; date of harvest, Sept. 4, 1935.
[2] Yield × sucrose percentage.
[3] *Exceeds the 5-percent point ($F=2\ 66$), ** exceeds the 1-percent point ($F=4\ 01$)
[4] Date of planting, Jan 25, 1936, date of application, Dec 18, 1935, date of harvest, Aug 1–5, 1936.
[5] * Exceeds the 5-percent point ($F=2.77$), ** exceeds the 1-percent point ($F=4\ 28$)

Increasing the application from 100 pounds to 200 pounds of nitrogen decreased the percentage of infection only slightly. Conceivably the heavy rainfall (about 15 inches) between the date of application, December 24, and the final date of planting, April 1, leached much of the available nitrates from the root zone, thus tending to equalize the two rates of application. On the other hand, the residual effect of calcium nitrate was at least equal to that of the other compounds—a fact which suggests that leaching did not greatly affect the results, since this form of nitrogen is more readily leached than are ammonium forms of nitrogen. The tops of the fertilized beets responded strikingly to the added nitrogen; the increase in root size is shown by the data in table 2.

The tonnage of all treated plots was greatly increased over that of the nontreated controls, and at harvest time the heavier rates of application still showed a somewhat lower percentage of sucrose.

The applications increased the gross sugar yield between 1,400 and 2,400 pounds per acre. Although calcium nitrate produced a higher yield than the other materials, the differences may not be significant. An average of the three 100-pound nitrogen applications shows a gain

of 1,783 pounds of sugar per acre, or a 38 percent gain over nontreated plots. The 200-pound applications show an average gain of 2,123 pounds of sugar per acre, or a 45 percent increase over the controls.

During 1936 another opportunity was provided to test how various forms and amounts of nitrogenous fertilizers affect the incidence of southern sclerotium rot in a field of relatively low fertility.

A 17-acre block in a field infested with *Sclerotium rolfsii* in Sutter County was selected for the experiment. Nitrogen-bearing fertilizers were broadcast with a fertilizer distributor on December 18, 1935, and the soil was then disked and harrowed in preparation for a seed-bed. Cyanamid was applied at the rate of 50, 100, and 200 pounds of nitrogen per acre; calcium nitrate at 50 and 100 pounds; ammonium sulfate and ammonium phosphate (16-20-0) at 100 pounds. The ammonium phosphate was included in order to learn whether phosphorus applied in addition to nitrogen would reduce the incidence of disease or increase the yield over that of nitrogen-treated plots. Each material was applied to three plots, 20 rows wide and 900 feet long (0.71 acre), and similar areas were left untreated. The seed was planted on January 25, and a good stand was obtained despite excessive rains that flooded part of the area and compacted the soil.

On March 12, a strong response of seedlings to the nitrogen fertilizers was exhibited in all plots except those receiving calcium nitrate and those in the nontreated area. After thinning, the calcium nitrate plots also showed a stimulation of leaf growth. The delay in response to this material can apparently be explained by the ease with which the nitrate is leached into the soil, though, as subsequent results indicate, little or no fertilizer material was leached beyond the ultimate root zone. All irrigation water was applied by sprinklers.

Diseased beets were counted in the four middle rows of each plot at intervals of 2 weeks from May 27 until harvest. All plots were harvested between August 1 and 5. To eliminate border effects, four rows were discarded between adjacent plots. When comparisons are made with the nontreated plots the incidence of disease is found to have been significantly reduced by each treatment except the lowest rate of Cyanamid, the ammonium sulfate, and the ammonium phosphate (table 2). Fifty pounds of nitrogen as calcium nitrate or 100 pounds as Cyanamid reduced the disease between 40 and 50 percent, whereas 100 pounds of nitrogen as calcium nitrate produced about 70 percent control, and 200 pounds as Cyanamid 75 percent control.

All treatments increased the average root weight; and although the percentages of sucrose were lower on the date of harvest, the tonnages from the fertilized plots were increased enough to provide an additional 1,000 to 2,100 pounds of gross sugar per acre.

As in the 1935 trials in an adjacent district, nitrogen from calcium nitrate produced a higher yield and held the disease at a lower level than the same amount of nitrogen from the other materials. Whether calcium nitrate is especially favorable for use on the heavier soils of northern Sutter County can be determined only by future trials. Its effect on the incidence of disease is especially significant because calcium nitrate is nontoxic to sclerotia or mycelium of *Sclerotium rolfsii*, as will be shown later in this report.

Since ammonium phosphate neither reduced the incidence of disease nor increased the yield as compared with the same amount of nitrogen from other sources, it may be concluded that at least under

the conditions of this experiment the addition of phosphorus was not beneficial.

The three foregoing experiments are typical of five made on soils of low fertility in that the percentage of infection was reduced by heavy applications of nitrogenous fertilizers, the yield of beets was strikingly increased, and the combined benefits from suppressing the incidence of disease and from crop stimulation considerably exceeded the cost of the treatments.

During the same years eight additional trials were conducted in fields which, judging by the plant growth, were abundantly supplied with available food elements. Detailed data from three such experiments follow.

TRIALS ON SOILS OF HIGH FERTILITY

In the hope of finding a soil amendment that could be applied to the soil to make possible the profitable production of sugar beets on land infested with *Sclerotium rolfsii*, four materials were applied. Manure at 10 tons per acre, hydrated lime at 5 tons per acre, and Cyanamid at 1,000 pounds per acre were incorporated in the soil on February 1. Ammonium sulfate was applied as a side dressing to the growing plants at 1,000 pounds per acre on May 16.

Five replications of each treatment and of nontreated plots, one eighty-second of an acre in size, were randomized in a linear series. Irrigation water was passed through 40-mesh screens in metal dams to prevent the introduction of sclerotia to treated plots. Throughout the growing season, the visibly infected plants in the middle four rows of each plot were counted at 10-day intervals. Table 3 presents data on the incidence of infection and yield from each treatment.

TABLE 3.—*Comparative effects of various soil amendments applied before planting on the incidence of infection and the yield of sugar beets growing in fertile soil infested with Sclerotium rolfsii, 1934* [1]

Treatment	Amount applied per acre	Infected beets	Average root weight	Sucrose content of beets	Acre yield of Beets	Acre yield of Gross sugar
	Pounds	*Percent*	*Pounds*	*Percent*	*Tons*	*Pounds*
Control	None	59 3	1 88	17 72	7 81	2,780
Hydrated lime [2]	10,000	45 5	1 80	17 40	10 92	3,820
Manure [2]	20,000	38 8	1 76	17 30	11 12	3,870
Ammonium sulfate [3]	1,000	50 1	2 00	15 32	10 76	3,270
Cyanamid [2]	1,000	24 8	1 86	15 56	16 83	5,220
Significant difference	{Odds 19.1 {Odds 99 1	14 6 20 1	29 .40	67 92	2.85 3 92	960 1,320
Calculated *F* value [4]		7 03**	89	25 37**	11 95**	8 22**

[1] Date of planting: Mar. 8, 1934.
[2] Applied before planting, Feb. 1.
[3] Applied as a side dressing, May 16
[4] *Exceeds the 5-percent point (F=3.01), **exceeds the 1-percent point (F=4 77).

The number of infected plants was considerably smaller in the Cyanamid-treated plots than in any of the others. Although manure and hydrated lime also proved somewhat beneficial, the side-dressing of ammonium sulfate apparently did not reduce the percentage of infection significantly below that of the nontreated plots.

Both the Cyanamid-treated and the ammonium-sulfate-treated plots showed a decided increase in green color and size of foliage be-

cause of the heavy nitrogen application, but the average root weight was not significantly increased over that of other plots. The sucrose percentage at harvest (August 11) showed a depression of approximately 2 percent in the ammonium sulfate and Cyanamid plots.

In total yield of sugar beets, the Cyanamid-treated plots showed a gain of 9 tons per acre over the nontreated plots and about 6 tons per acre over the other treatments. This gain was due almost entirely to the prevention of infection, since the differences in average root weight were not significant. In gross sugar yield there was a gain of 2,400 pounds in the Cyanamid-treated over the nontreated plots and a gain of approximately 1,400 pounds over the lime- and manure-treated plots. The same amount of nitrogen applied as a side dressing of ammonium sulfate failed to produce a significant improvement in survival of plants or in yield over the nontreated plots.

Under the conditions of this experiment, Cyanamid at the rate of 1,000 pounds per acre considerably reduced the percentage of infection and made possible the production of a profitable crop, whereas the other materials used (manure, lime, and ammonium sulfate as a side dressing) did not produce sufficient improvement to justify their use.

The most striking features of the results are (1) the suppression of infection by heavy application of Cyanamid even though no increase in root size occurred, and (2) the failure of equal amounts of ammonium sulfate to produce similar effects when sidedressed at a shallow depth comparatively late in the growing season. Most likely the ammonium sulfate was leached into the soil too late to produce the effects obtained by applying the Cyanamid before planting. Supplementary observations and investigations apparently preclude the possibility of a specific toxic effect of Cyanamid on *Sclerotium rolfsii* in field applications.

One experiment during 1935 was conducted on a very fertile plot of land adjoining the Sacramento River on Merritt Island in Yolo County. Comparisons were made of applications of 50 to 200 pounds of nitrogen per acre as anhydrous ammonia, ammonium sulfate, or calcium nitrate dissolved in the surface irrigation water. Eight treatments were each replicated six times on 1/20-acre plots in randomized blocks. Because of the high water level in the Sacramento River, the soil in part of the experimental area was saturated, and some water flowed across the surface of the soil from seepage under the protecting levee during March and April. As a result, only two of the six blocks provided conditions favorable for the experiment.

Since the data (table 4) consist of averages from only these two blocks, no attempt has been made to calculate the significance of differences.

Judging from the results, the reduction of infection from *Sclerotium rolfsii* was in proportion to the amount of nitrogen applied, and the form of nitrogen exerted little influence. It should be noted that in this field there was no evidence that the fertilizers increased the size of the roots. All increases either in tonnage or in gross sugar were apparently due to the reduction of infection by *S. rolfsii*. The large average root weights of the nontreated beets may have been due to the fact that large numbers of plants were killed by the fungus early in the growing season and that the survivors were relatively free from competition with adjacent plants; or possibly the smaller beets in the nontreated

plots were infected at an early date, thus permitting the survival of only the larger plants.

TABLE 4.— *Comparative effects of various nitrogenous fertilizers applied in the irrigation water on the incidence of infection and the yield of sugar beets growing in fertile soil infested with Sclerotium rolfsii, 1935*

Treatment	Fertilizer applied per acre	Nitrogen applied per acre	Infected beets	Average root weight	Sucrose content of beet	Acre yield of —	
						Beets	Gross sugar [1]
	Pounds	*Pounds*	*Percent*	*Pounds*	*Percent*	*Tons*	*Pounds*
Control	None	None	72 7	2 12	17 3	6 26	2,170
Ammonia .	62	50	60 0	1 80	16 8	8 42	2,840
Ammonium sulfate.	245	50	53 7	1 75	16 9	9 80	3,285
Ammonia	124	100	43 8	1 67	17.4	12 66	4,360
Ammonium sulfate .	490	100	42 3	1 84	16.3	13 15	4,265
Ammonia	248	200	23.0	1 66	15 7	18 05	5,670
Ammonium sulfate..	980	200	30 5	1 69	17 1	14 69	5,000
Calcium nitrate...	1,290	200	33 4	1.72	16 7	13 94	4,640

[1] Yield×sucrose percentage.

During 1937 two series of plots were established in a field near Knights Landing, Sutter County, to show the effect of moderate applications of ammonium sulfate or anhydrous ammonia on the incidence of southern sclerotium rot in a relatively fertile field. An area was selected which, according to soil samples, showed a population of about 65 sclerotia per square foot, sufficient to produce an infection of 5 to 8 percent (*13*) by August 1.

In one part of the field, ammonium sulfate was applied at the rate of 100 pounds of nitrogen per acre to six 16-row plots of sugar beets alternating with nonfertilized strips of the same width. One-half of the material was applied with the seed on April 5; the other half was side dressed on June 4, just before the first irrigation. Since very little rain fell between these dates, presumably little or none of the fertilizer was in solution and available to the plants until after the irrigation.

In another part of the same field, a similar series of plots was arranged in cooperation with the Association Laboratories of Anaheim, Calif. In this series. five 16-row plots were fertilized with anhydrous ammonia applied in the first irrigation at the rate of 100 pounds of nitrogen per acre, and an equal number of adjacent plots were left unfertilized.

Before the first irrigation, and at intervals of 2 weeks thereafter, the number of infected beets in each plot was determined. The rate of increase in the fertilized and nonfertilized plots is shown in figure 3, *A* and *B*. These infection curves show that both ammonium sulfate and ammonia strikingly reduced the percentage of infection. This effect was noticeable in the ammonia-treated plots within 2 weeks after the application, but a longer period was required in the ammonium sulfate plots. Since these two materials have in previous trials produced similar results when both were dissolved in the irrigation water, apparently the differences in this case were due mainly to the method of application, the side-dressed material being less quickly available to the plants.

In the same way the ammonia-treated plots showed a striking stimulation in top growth soon after the application, whereas the

ammonium-sulfate-treated plots gave a slower and less evident response. Table 5 shows the differences in the average percentage of infection and yield of the two series of plots.

The ammonium-sulfate-treated plots showed an average increase of 2.8 tons per acre and about 700 pounds of sugar per acre over the nontreated plots. The sucrose percentage appeared slightly lower in the fertilized plots, but the difference was not statistically sig-
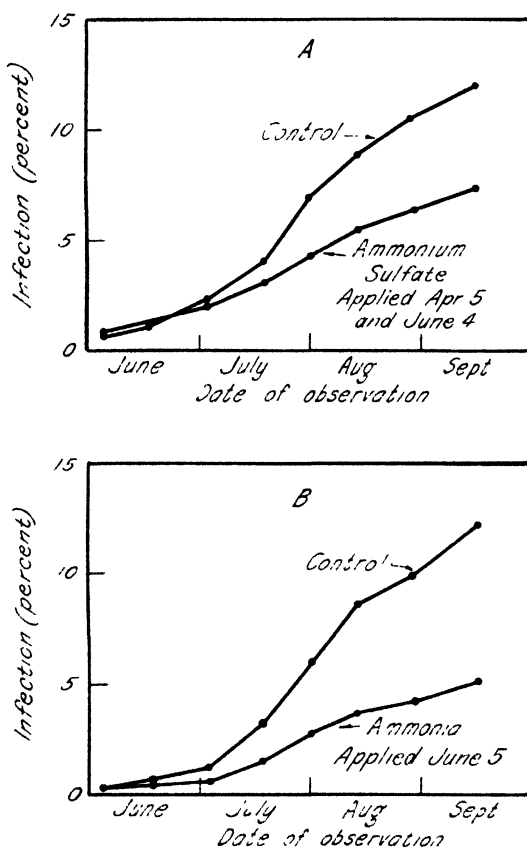
FIGURE 3.—Effect of nitrogenous fertilizers on infection of sugar beets by *Sclerotium rolfsii*. *A*, Ammonium sulfate side-dressed at time of planting and just before first irrigation in comparison with nonfertilized plots; *B*, anhydrous ammonia dissolved in first irrigation water. Both materials applied at the rate of 100 pounds of nitrogen per acre.

nificant. Because of the variation in the sucrose percentage of the plots, the increase in yield of sugar per acre of the fertilized over the nonfertilized plots appears not to be statistically significant, though the yield in tons per acre shows significant differences. The ammonia-treated plots showed an average increase of 4.7 tons of beets per acre and 1,200 pounds of sugar per acre over the nontreated plots. As in the other series, the apparent reduction in sucrose percentage was not significant. Since the two series of plots were located in different parts of the field, the increases in yield cannot be compared directly. Furthermore, the apparent superiority of the anhydrous ammonia is

probably due, at least in part, to the time and method of application, as previously mentioned.

TABLE 5.—*Comparative effects of ammonium sulfate applied to the soil and anhydrous ammonia applied in the irrigation water on the incidence of infection and the yield of sugar beets growing in soil infested with Sclerotium rolfsii, 1937*

Series and treatment	Nitrogen applied per acre	Infected beets	Sucrose content of beets	Acre yield of—	
				Beets	Gross sugar [1]
Series 1	*Pounds*	*Percent*	*Percent*	*Tons*	*Pounds*
Control	None	11 97	15 36	18 48	5, 692
Ammonium sulfate	100	7 30	15 09	21 28	6, 397
Significant difference, 19 1		3 05	2 38	2 14	1, 170
Series 2					
Control	None	12. 20	16 91	21. 81	7, 380
Ammonia	100	5 06	16 17	26 53	8, 600
Significant difference, 19 1	....	5 00	1 11	3 77	1, 010

[1] Yield×sucrose percentage.

DISCUSSION OF EXPERIMENTS ON NITROGEN FERTILIZERS IN RELATION TO SOUTHERN SCLEROTIUM ROT

During the 4 years from 1934 to 1937 inclusive, 13 separate replicated experiments were conducted in various parts of the Sacramento Valley to determine the relation of nitrogenous fertilizers to the incidence of southern sclerotium rot of sugar beets. Table 6 summarizes the results.

TABLE 6.—*Summary of 13 experiments on the relation of nitrogenous fertilizers to the incidence of Sclerotium rolfsii infection on sugar beets*

Treatment	Trials	Relative amount of infection [1]	
		Range	Mean
	Number	*Percent*	*Percent*
Control	13		100
50 pounds of nitrogen per acre as—			
Ammonia	1		82 5
Ammonium sulfate	2	61 9–73 9	67 9
Calcium nitrate	1		59 1
Cyanamid	1		81 1
Average for 50 pounds of nitrogen per acre			71 8
100 pounds of nitrogen per acre as—			
Ammonia	4	19 7 60 2	40 8
Ammonium sulfate	8	43 8–64 4	53 6
Calcium nitrate	6	21 4–61 0	36 2
Cyanamid	5	35 7 80 9	54 7
Average for 100 pounds of nitrogen per acre			46 3
200 pounds of nitrogen per acre as—			
Ammonia	3	25 3 31. 6	28 0
Ammonium sulfate	6	9 5–[2] 84. 5	41 1
Calcium nitrate	4	16. 1–45 9	33. 3
Cyanamid	5	25. 0 51 9	39 0
Average for 200 pounds of nitrogen per acre			35 4

[1] The relative amount of infection in each experiment is equal to 100 times the ratio of the percentage of infection in the fertilized plots to that in the nonfertilized plots.
[2] Failure of control was apparently due to shallow side dressing of the ammonium sulfate late in the growing season.

There was considerable variability in the average percentage of infection in the nontreated plots of the different experiments. For direct comparison, the results must be converted to a relative basis. Accordingly, the average percentage of infection in the nontreated plots in each experiment has been considered as 100, while the percentage of infection in the fertilized plots in the same experiment is represented as a given percentage of that amount. For example, the average of all experiments in which 50 pounds of nitrogen was added shows 71.8 percent as much disease as in the nontreated plots of the same experiments. In the same way 100 pounds of nitrogen per acre showed an average of 46.3 percent as much disease as in the control plots; 200 pounds of nitrogen, 35.4 percent. The degree of control obtained by using the same material in different experiments usually showed greater differences than were obtained by the use of different forms of nitrogen in the same experiment. No convincing proof has, therefore, been obtained that one source of nitrogen surpasses another for use over the wide range of conditions covered by these experiments. In all cases, the greater the dosage of a given material, the greater the degree of control obtained, although occasionally the differences were not statistically significant.

In some experiments the fertilizers were broadcast in advance of planting; in others the materials were dissolved in the irrigation water; and in three cases the fertilizers were applied as a side dressing to the growing crop. Satisfactory results have been obtained with each of these methods of application, and the results show no consistent differences in favor of any certain time or method. In general, however, applications that promoted the most vigorous vegetative growth of the beets were most effective in retarding the infection by *Sclerotium rolfsii*. For satisfactory results, the applications must be made before any large percentage of beets has been infected.

Planting of heavily infested fields with sugar beets in the hope of controlling the disease with nitrogen fertilizers is to be discouraged; judging from the results obtained in the present experiments, no more than a 50 to 75 percent control can be expected. In lightly or moderately infested fields the percentage of infection has often been kept low, but the increase in yield due to disease control will seldom balance the cost of the material. Economical use of this method of control is limited, theretofore, to fields in which nitrogen fertilizers increase the yield of sugar per acre enough to equal at least the cost of the fertilizer and the other costs incidental to its usage.

TOXICITY OF NITROGEN COMPOUNDS TO *SCLEROTIUM ROLFSII*

LABORATORY STUDIES

In view of the striking reduction of infection on sugar beets produced by nitrogenous fertilizers, it is interesting to consider how these materials affect the causal fungus. As shown by laboratory studies previously reported (*1, 2, 12*), anhydrous ammonia in solution (ammonium hydroxide) is very toxic to sclerotia and mycelium of *Sclerotium rolfsii*, being of the same order of fungicidal power as formaldehyde.

Salts of ammonia such as ammonium sulfate and ammonium phosphate have also proved toxic to mycelium of the fungus, but only in alkaline solutions. In these trials, units of *Sclerotium rolfsii*

mycelium were obtained by germinating surface-sterilized sclerotia upon filter paper moistened with standard beef broth diluted to one-twenty-fifth strength with water. After the mycelium had been allowed to grow for 4 days at 30° C. the filter paper bearing the mycelium was cut into small squares with sides of 0.5 cm. Twenty of these squares, constituting a test sample, were immersed in the test solutions in shell vials for definite periods at room temperature. They were then washed for not less than 5 minutes in tap water and plated on moist nonsterilized peat soil that had been passed through a 40-mesh screen. It was found that solutions of ammonium sulfate with an ammonium content equivalent to 82.4 millimols of ammonia
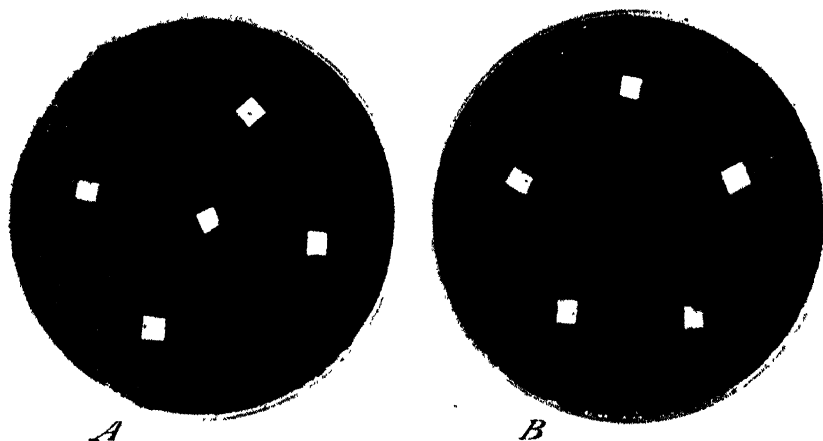
FIGURE 4.—Effect of calcium carbonate on the toxicity of ammonium sulfate to mycelium of *Sclerotium rolfsii* as indicated by: (*A*), Absence of growth after immersion in a solution containing 1,400 p. p. m. of ammonium sulfate in addition to an excess of calcium carbonate. (*B*) Growth of mycelium after immersion in a saturated solution of calcium carbonate at pH 8.4 for 24 hours. Similar growth followed immersion in a 1,400 p. p. m. solution of ammonium sulfate. The substratum was nonsterilized peat soil.

(NH_3) per liter (pH 6.0) and solutions of monammonium phosphate with the same ammonia content (pH 4.5) were nonlethal to mycelium of *Sclerotium rolfsii* immersed in them for 5 days. In the same way a saturated solution of calcium carbonate ($CaCO_3$) was nonlethal at pH 8.4 (quinhydrone electrode). The addition of calcium carbonate to the solution of ammonium sulfate containing the equivalent of 41.2 millimols of NH_3 and giving a pH of 7.8 resulted in the death of 96 to 100 percent of the mycelial units (fig. 4). Solutions of ammonium bicarbonate and dibasic ammonium phosphate, with a pH of 7.5 with equivalents of 17.8 and 14.7 millimols of ammonia respectively, were also lethal within 5 days to immersed mycelium. These results suggest that the toxicity of ammonium sulfate is due to the release of NH_3 by hydrolysis in an alkaline solution. In alkaline soils applications of anhydrous ammonia or ammonium sulfate may result in conditions similar to those in the solutions buffered with calcium carbonate, and thus it appeared possible that ammonia in

solution might be the effective agent in reducing the extent of infection by inhibiting fungus growth.

Calcium cyanamide in aqueous solution appeared to be only mildly toxic to this fungus. Mycelium was killed, however, after immersion for 120 hours in a solution containing 1,500 p. p. m. of dry commercial Cyanamid.

Calcium nitrate exhibited no fungicidal effect on mycelium or sclerotia of *Sclerotium rolfsii;* and in one trial there was some evidence that the fungus growth was stimulated by applications of this material.

The formation of NH_3 from calcium nitrate in toxic amounts by denitrification processes in well-aerated soils seems highly improbable in view of the experience of E. L. Proebsting (unpublished data), who has found that in orchard soils to which calcium nitrate was added the NH_3 content was not detectable by ordinary methods of analysis.

FIELD TRIALS

If, as has been suggested, the partial control of the southern sclerotium root rot is due to the toxicity of ammonium compounds or Cyanamid, then field trials should demonstrate an appreciable reduction in number or viability of sclerotia after such applications.

In one series of trials, diluted ammonium hydroxide was applied to the surface of duplicate plots of heavily infested soil at the rate of 3,000 pounds of ammonia per acre. Ten days later sclerotia recovered from the ammonia-treated areas averaged 90.5 percent germination, whereas those recovered from the nontreated areas averaged 86.7 percent germination.

In another series of plots Cyanamid was broadcast on the surface of heavily infested plots at the rate of 945 pounds (200 pounds of nitrogen) per acre and mixed with the soil by disking. Soil samples were collected from duplicate plots at intervals of 33, 60, and 115 days after treatment. Table 7 shows the number of sclerotia recovered from treated and nontreated areas, together with the average germination of the sclerotia.

TABLE 7.— *Germinability of sclerotia recovered from field soils after treatment with Cyanamid*

Period exposed in soil (days)	Rate of application of Cyanamid per acre	Sclerotia recovered per 500 gm of soil	Average germination
	Pounds	*Number*	*Percent*
33	None	91	95
	945	109	80
60	None	67	87
	945	71	92
115	None	89	99
	945	73	91

Evidently, neither anhydrous ammonia nor Cyanamid applied to the surface of infested soils produced any appreciable toxic effects on the sclerotia under the conditions of these trials. These observations do not, however, preclude the possibility that the chemicals may have retarded germination of sclerotia, inhibited growth of mycelium, reduced its infective ability, or even destroyed the mycelium of the causal fungus within the soil.

DISCUSSION

Evidence has been presented to show that applications of anhydrous ammonia, ammonium sulfate, Cyanamid, or calcium nitrate will appreciably reduce the amount of southern sclerotium root rot in sugar beets.   In solution the first three materials exhibit various degrees of toxicity to the mycelium of *Sclerotium rolfsii*, whereas at similar concentrations calcium nitrate is nonlethal.   In field soils excessive applications of anhydrous ammonia and Cyanamid have failed to kill sclerotia of this fungus.

Ammonium compounds in alkaline media liberate sufficient NH_3 to provide a low degree of toxicity to mycelium, and this phenomenon may explain their partial control of sclerotium root rot.   The fact that calcium nitrate, which is itself nonlethal to mycelium and apparently does not liberate NH_3 in toxic amounts, is equally effective in reducing sclerotium rot suggests that this reduction is probably not due to the toxicity of nitrogen compounds to the causal fungus.

While there is evidence against the acceptance of ammonia as a toxic agent in these field trials, there is information from investigations by Higgins (5) and Krüger (10) which suggests that the presence of ammonia or other basic substances may so affect the metabolic activity of the fungus as to reduce its ability to injure or invade the tissue of the sugar beet.   Both Higgins and Krüger agree concerning the importance of oxalic acid produced by the fungus in the processes of infection.   Higgins has also shown that substrates of high nitrogen content result in the formation of considerable amounts of ammonium oxalate which he found to be much less toxic than oxalic acid.

A second possible explanation for the reduced infection is that it may be due to a physiological or anatomical resistance induced in the plants.   There appeared to be a close correlation between nitrogen stimulation to sugar beets (as exhibited by leaf and root growth) and the retardation in the development of the disease.   The effect of nitrogenous fertilizers in reducing the disease was nearly as pronounced in fields of high fertility as in fields of low fertility, which would suggest that any resistance contributed to the sugar beet is associated with a luxury consumption of nitrogen on the part of the host rather than with the correction of a deficiency as usually understood.

The possibility remains that the application of large amounts of nitrogen compounds may have stimulated certain micro-organisms to pronounced multiplication and activity and in some way this biologic change suppressed *Sclerotium rolfsii*, thus preventing the usual amount of infection.

Neither the writers' investigations nor those of others provide an adequate basis for evaluating the relative importance of the above hypotheses.

SUMMARY

Applications of nitrogenous fertilizers in field plots have consistently reduced the percentage of *Sclerotium rolfsii* infection on sugar beets.   Ammonium sulfate, anhydrous ammonia, calcium nitrate, and Cyanamid proved equally effective when equivalent amounts of nitrogen were supplied under favorable conditions.   According to the averages of all trials, 50 pounds of nitrogen per acre reduced infection

by about 28 percent, 100 pounds by 54 percent, and 200 pounds by 65 percent, as compared with nonfertilized areas. Yields were increased by these treatments both because of the greater number of disease-free beets and because of the stimulation of growth produced by the fertilizers in most of the fields. Economical use of this method of control is limited to areas or fields where sugar beets respond to nitrogenous fertilizers.

According to laboratory trials, low concentrations of ammonia in aqueous solution are toxic to mycelium and sclerotia of *Sclerotium rolfsii*. Ammonium sulfate in alkaline solutions is also mildly toxic to mycelium. Calcium nitrate in solutions of similar concentrations was nontoxic to mycelium or sclerotia.

The fact that these three materials, differing in toxic relations, were equally effective in preventing infection from *Sclerotium rolfsii* suggests that the control obtained may have been due to factors other than ammonium toxicity.

Heavy applications of anhydrous ammonia and Cyanamid to naturally infested soils failed to destroy the sclerotia or to reduce their viability. The effects upon mycelium in the soil were not determined.

To explain the partial control of southern sclerotium rot on sugar beets by nitrogenous fertilizers, it is suggested that changes in the metabolism of the causal fungus may reduce its growth or pathogenicity; that resistance of the host may be increased by alteration of its metabolism and anatomy; or that there may be a suppression of the causal organism because of a change in the balance of micro-organisms in the soil.

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EFFECT OF METAL CONTAINERS ON THE ANAEROBIC FERMENTATION OF CORNSTALK FLOUR BY THERMOPHILES[1]

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INTRODUCTION

The study reported in this paper was undertaken to ascertain the effect of metal containers on the continuous thermophilic anaerobic fermentation of cornstalk flour by a methane-producing seed. It was necessary to obtain this information before large metal tanks were built for the continuous anaerobic fermentation of fibrous farm wastes.

Much has been written on the oligodynamic action of heavy metals on micro-organisms, but the literature will not be reviewed here since Buchanan and Fulmer have given an excellent review in their book (*3*).[3]

MATERIALS EMPLOYED

Cornstalk flour was used as the test material because the authors had used it in previous experiments (*5, 6, 7*), and were familiar with the manner in which it is digested. It was prepared by wet-grinding the stalks in a rod mill and drying. Most of this flour passed a 200-mesh sieve.

The thermophilic methane-producing seed was originally developed from sewage sludge, as described in a previous publication (*5*). Before it was used, it was passed through a screen with circular openings 1 mm. in diameter, and all solids remaining on the screen were discarded. A preliminary analysis of the seed was made, and sufficient urea was added to bring the ammonia content of the mixture to from 500 to 800 p. p. m.

The fermentation apparatus consisted of wide-mouth glass bottles and metal containers. The glass bottles, used for controls, were fitted with three-hole rubber stoppers (No. 14), with outlets for the removal of samples for pH determinations, for the addition of chemicals when necessary, and for a gas exit. Anaerobic conditions were maintained by means of a water seal. Details of the construction of the glass fermentation apparatus are given in a previous publication (*5*). The gas was collected and measured in separate glass bottles. The 9-liter metal containers were made of copper, galvanized iron, black sheet iron, or stainless steel (*18–8*).[4] They were cylindrical with tapering tops, and the openings held the same size rubber stoppers as were used with the glass bottles. No solder was exposed on the inside of the containers. All details of the procedure were carried out the same in the metal as in the glass containers.

Previous work in this laboratory (5) had shown that approximately half as much time was required to produce a unit quantity of gas from cornstalk flour at 50° to 55° C. as was required at 28° to 30°; therefore it was decided to employ the higher temperature in this experiment.

Five consecutive fermentations were run in all the containers except the copper, in which only four were run. In each run the following containers were used: Glass (seed only), glass (seed+cornstalk flour), galvanized iron, copper, black sheet iron, and stainless steel (18 8). The fermentations in the glass containers were controls. A detailed description of the preparation of the mixtures and the analyses for run 1 are given below. With the exception noted, the treatment of the mixtures was the same in all five runs.

PROCEDURE

Run 1.—With the exception of the seed control in the glass container, to which no cornstalk flour was added, all the fermentation mixtures were the same. They were prepared by placing 3 liters of seed, 1 liter of tap water, 65 gm. of cornstalk flour, and 2.5 gm. of urea in the container. The rubber stopper was tightly inserted, held securely with stovepipe wire, and sealed with shellac and sealing wax. The gas evolution tube was connected to a water seal, and the mixture was incubated at 50° to 55° C. for 22 days. The pH was checked frequently, and when necessary was adjusted to insure the optimum reaction (pH 7.0 to 8.0). A daily record was made of the gas produced. The volume of gas was measured as previously described (5), and the gas was analyzed for carbon dioxide, hydrogen, and methane by means of a modified Orsat apparatus (4).

At the termination of the 22-day fermentation period, all the containers were removed from the incubator, and the volume of the residue in each was measured. After thorough mixing, 1-liter aliquots were removed for analysis, and the remainder was returned to the container to serve as seed for the next run. A small part of one aliquot was used for a "wet" analysis; the remainder was evaporated to dryness on a steam hot plate, ground to pass a 30-mesh screen, and analyzed.

The following determinations were made on the wet samples: (1) Total solids; (2) ash-free solids (loss on ignition); (3) ash; (4) specific gravity; (5) ammonia nitrogen; (6) Kjeldahl nitrogen; and (7) metals (iron, copper, and zinc) according to the methods of the American Public Health Association and the American Water Works Association (1).

The following determinations were made on the dried residues: (1) Total solids; (2) ash-free solids (loss on ignition at about 600° C. for 1 hour) by the American Public Health Association and American Water Works Association method (1); (3) pentosans by the Association of Official Agricultural Chemists' method (2); (4) cellulose by the Norman and Jenkins' method (8) (with corrections made for ash and pentosans); (5) lignin by the Norman and Jenkins' method (9, 10), substituting, however, 2-percent acid hydrolysis for 2 hours for the 5-percent hydrolysis normally used. (Duplicate lignin samples were run; one was ashed and the other was analyzed for Kjeldahl nitrogen by the A. P. H. A. and A. W. W. A. method (1).) A correction for ash was made, but no correction for nitrogen was applied, inasmuch as

Norman and Jenkins (*10*) have shown that the usual protein factor is not reliable); (6) Kjeldahl nitrogen by the A. P. H. A. and A. W. W. A. method (*1*); and (7) iron, copper, and zinc as follows: Iron by the thiocyanate method; copper by the sodium diethyldithiocarbamate method; and zinc by the ferrocyanide method as outlined by the American Public Health Association and American Water Works Association (*1*).

Run 2.—To the residue in each of the containers from run 1 were added 65 gm. of cornstalk flour, 1 liter of tap water, and ½ gm. of urea. No cornstalk flour was added to the seed control. The mixtures were incubated for 23 days. The rest of the procedure was the same as that outlined for run 1.

Run 3.—To each of the residues from run 2 were added the same quantities of cornstalk flour, water, and urea as were added in run 2. No cornstalk flour was added to the seed control. The mixtures were incubated for 25 days. At the end of the run, only a small sample (100 cc.) was removed for a "wet" analysis.

Run 4.— To each of the residues from run 3 were added 65 gm. of cornstalk flour and 1 gm. of urea. No water was added. No cornstalk flour was added to the seed control. The mixtures were incubated for 30 days. At the end of the run only a small sample was removed for a "wet" analysis.

Run 5.—To each of the residues from run 4 were added 65 gm. of cornstalk flour and 2 gm. of urea. No water was added. No cornstalk flour was added to the seed control. The mixtures were incubated for 60 days. At the end of the run a complete analysis was made (as in runs 1 and 2). The complete analysis of run 5 really was an analysis of the combined runs 3, 4, and 5.

RESULTS

As very little gas was produced in the seed control and the range of pH was optimal (pH 7.0 to 8.0), this part of the experiment will not be discussed further. In compiling the figures for the fermentations carried out in the other containers, a correction was made for the seed control.

For convenience the results are presented by "runs."

RUN 1

The volumes of gas produced are shown graphically in figure 1. The percentage decomposition of the constituents of the cornstalk flour, the composition of the gas, the pH range of the mixtures, and other pertinent data are presented in table 1. Table 6 shows the quantities of the metals in the mixtures at the end of the run.

In order to judge better the relative effects of the various metal containers on the fermentation, it was decided to rate the containers numerically on the basis of (1) the quantity of gas produced; (2) the methane content of the gas and the percentage decomposition of the (3) ash-free solids, (4) pentosans, (5) cellulose (corrected for ash and pentosans), and (6) lignin of the cornstalk flour. The following numerical values were assigned: 5 for maximum activity, 4 for next high, 3 for the third, 2 for the fourth, and 1 for the minimum activity. In case any two or more containers were rated approximately the same, each was given an equal share of the combined points for the two or more places in question. The maximum score obtainable would be 30.

TABLE 1.—*Decomposition of constituents in cornstalk flour fermented anaerobically for 22 days at 50° to 55° C. and gas produced, run 1*

Item	In original material			After fermenting for 22 days in—				
	Seed	Corn-stalk flour	Total	Glass con-tainer	Gal-van-ized iron con-tainer	Cop-per con-tainer	Sheet-iron con-tainer	18–8 stain-less steel con-tainer
Constituents:								
Total solids ... _ grams..	63.25	62 97	¹128 72	87 65	94 53	123 32	89.01	85.70
Ash-free solids .. do....	41 36	60.74	¹104 60	62.22	67 81	95 03	61.68	58.90
Pentosans do ..	1.54	20 58	22 12	5 41	6 50	17 08	5 63	4.64
Crude cellulose (ash-free) do ...	3 30	29.69	32.99	9 76	10 50	23.42	8.63	7.01
Cellulose (corrected ²) .. _ do ...	2 63	17.61	20 24	7 48	7 89	18.75	6.68	5 40
Lignin. --- ------ do .	21 81	10 25	32 06	27 93	29 66	31 74	27 48	28 52
N² in lignin .percent.	2 00	0.66	--------	1 95	1 98	1.73	2.04	1.83
Decomposition of constituents in corn-stalk flour:								
Total solids ... ... percent..				65.35	54.30	8.58	63 15	68 45
Ash-free solids ... . do -				69 75	60 90	15 75	70.55	75.20
Pentosans ...... do				81.20	75 90	24 52	80.20	85 00
Crude cellulose (ash-free) do...				78 20	75 80	32.22	81.80	87 50
Cellulose (corrected ²).. do _				72 50	70 10	8 46	76 97	84 25
Lignin . do				40 30	23 45	3 12	43 70	34 55
Gas produced under 30 inches of Hg at 60° F.·								
Total . cc.				22,835	21,475	3,795	20,605	23,135
Per gram of cornstalk flour do ...				363	341	60	327	367
Per gram of ash-free solids in corn-stalk flour .... cc....				376	354	63	339	381
Per gram loss of ash-free solids cc....				539	584	397	480	506
Average composition of gas·								
CO₂ ---- --------- percent..				36.1	28 5	10 1	25.4	33 8
H₂ . _do				2 1	2 0	2 2	4 3	3.3
CH₄..... do				45.4	43 3	0	49 6	45 7
pH range ..				7 5–7 9	7 5–7.8	7 5–8 0	7 5–7 8	7.4–7.8

¹ Includes 2 5 gm. of urea.
² Corrected for pentosans and ash.
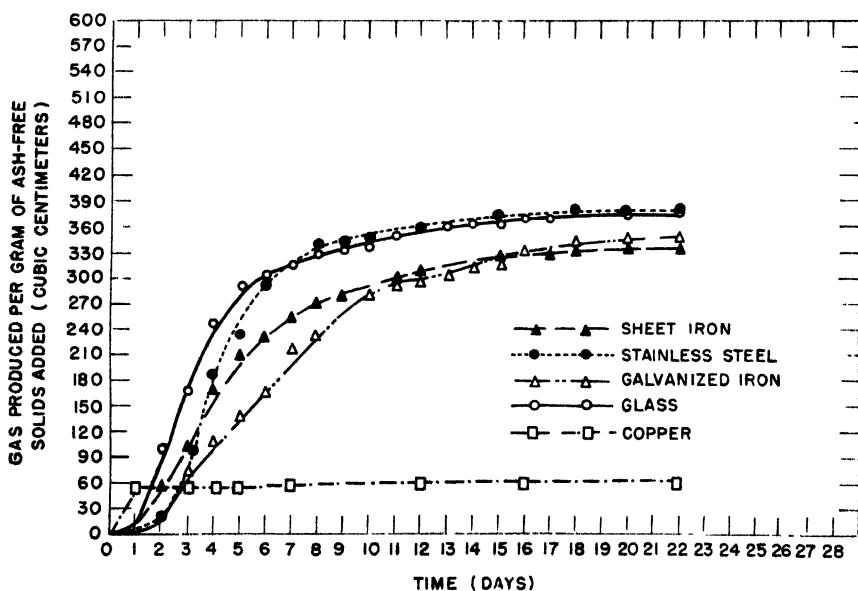
FIGURE 1.—Quantity of gas produced (cumulative) from a fermentation mixture of cornstalk flour and active methane-producing seed incubated at 50° to 55° C. in glass and various metal containers. Run 1.

The following example illustrates this method of evaluation: The volumes of gas produced (fig. 1) in the stainless-steel container and in the glass container were nearly the same but were greater than those produced in the other containers. Therefore, the numerical value given is $\underline{5+4}$ or 4.5 points for each. Similarly, the methane
2
values (table 1) for the stainless steel and for the glass containers were the same but were less than that for the sheet iron. The value $\underline{4+3}$
2
or 3.5 is given for each. The ash-free solids losses (table 1) for the stainless steel, the glass, and the sheet-iron containers were fairly

FIGURE 2.—Quantity of gas produced (cumulative) from a fermentation mixture of cornstalk flour and active methane-producing seed incubated at 50° to 55° C. in glass and various metal containers. Run 2.

close and were greater than those for the other containers. The value
$\underline{5+4+3}$ or 4 is given for each. The pentosan losses (table 1) for the
3
stainless steel, the glass, and the sheet-iron containers were fairly close and were greater than those for the other containers. The value
$\underline{5+4+3}$ or 4 is given for each. The cellulose loss (table 1) was great-
3
est for the stainless-steel container, and the value 5 is given. Lignin losses (table 1) were greater in the sheet-iron and the glass containers than in the stainless-steel container so the value 3 is given for the stainless steel. On this basis the containers were scored as follows: Stainless steel, 24; glass, 22.5; sheet iron, 21.5; galvanized iron, 16; and copper, 6.

<center>RUN 2</center>

The percentage decomposition of the constituents of the cornstalk flour, the composition of the gas, the pH range of the mixture, and other pertinent data are presented in table 2. The volumes of

the gas are shown graphically in figure 2.   Table 6 gives the quantities of the metals in the mixtures at the end of the run.

The scoring, done on the same basis as in run 1, was as follows: Glass, 28; stainless steel, 21; sheet iron, 17.5; galvanized iron, 13.5; and copper, 10.

TABLE 2.— *Decomposition of constituents in cornstalk flour fermented anaerobically for 23 days at 50° to 55° C. and gas produced, run 2*

Item	At start in—					After fermenting for 23 days in—				
	Glass container	Galvanized iron container	Copper container	Sheetiron container	18–8 stainlesssteel container	Glass container	Galvanized iron container	Copper container	Sheetiron container	18–8 stainless steel container
Constituents										
Total solids ... grams	1 128 34	1 133 95	1 159 39	1 129.50	1 127 76	87.13	106 53	142 97	93 03	92 10
Ash-free solids ... do	1 107.29	1 111.80	1 132 35	1 107.00	1 105.42	62 12	80 20	111 24	64 89	66 50
Pentosans ... do	24 58	25.43	33 37	24 76	24 06	6 54	12 24	26 74	7 27	7 06
Crude cellulose (ash-free) grams	36.91	37 52	41.21	36 09	34 95	10 33	20 46	31 71	12 56	10 51
Cellulose (corrected 2) do	23 14	23 50	31.64	22 56	21.66	8 10	14 75	25 58	8 51	7 81
Lignin do	30 92	32 38	34.00	30.63	31.65	26 49	29 13	28 58	28 02	28 65
N_2 in lignin percent						1.84	1.80	1 59	2 07	1 97
Decomposition of constituents in cornstalk flour:										
Total solids percent						65 45	43 55	26 10	57 95	56 55
Ash-free solids do						74 45	52 05	34 75	69 50	64 00
Pentosans do						87 60	64 10	32 21	85 20	82 70
Crude cellulose (ash-free) percent						89 45	57.45	31 92	75 80	82 45
Cellulose (corrected 2) do						85 45	49.60	34.40	79 80	78 55
Lignin do						43 25	31 70	53 00	25 45	29 25
Gas produced under 30 inches of Hg at 60°F.:										
Total cc						25,115	11,820	390	16,780	22,570
Per gram of cornstalk flour cc						399	188	6 2	266	358
Per gram of ash-free solids in cornstalk flour cc						414	195	6 4	276	372
Per gram loss of ash-free solids cc						556	374	18 5	399	580
Average composition of gas										
CO_2 percent						39 1	24 1	2 0	29 9	33 2
H_2 do						1.9	1.5	2 9	1 8	2.2
CH_4 do						44 7	31 1	0	23 5	39 6
pH range						7 5–7 6	7 0–7 6	7 6–8 2	7 6 8 0	7 5 7 8

[1] From 65 gm of cornstalk flour, 0 5 gm of urea, and bacterial seed
[2] Corrected for pentosans and ash.

RUNS 3, 4, AND 5

The volumes of gas are shown graphically in figures 3, 4, and 5. The percentage decomposition of the ash-free solids of the cornstalk flour, the pH range, the gas composition, and other pertinent data are recorded in table 3.   Table 6 gives the quantities of the metals in the mixtures at the end of the runs.   The scoring of the containers for these three runs was done on the basis of the quantity of gas produced, the methane content of the gas, and the percentage decomposition of the ash-free solids of the cornstalk flour.   In run 3, with 15 as the highest score possible, the containers were rated as follows: Glass, 13; stainless steel, 12; sheet iron, 9; galvanized iron, 8; and copper, 3.   In run 4, likewise with 15 as the highest score possible, the containers were rated as follows: Glass, 13; stainless steel, 12; sheet iron, 11; galvanized iron, 6; and copper, 3.   In run 5, with 12 as the highest possible score, the containers were rated as follows: Stainless steel, 10.5; glass, 8.5; sheet iron, 8; and galvanized iron, 3.
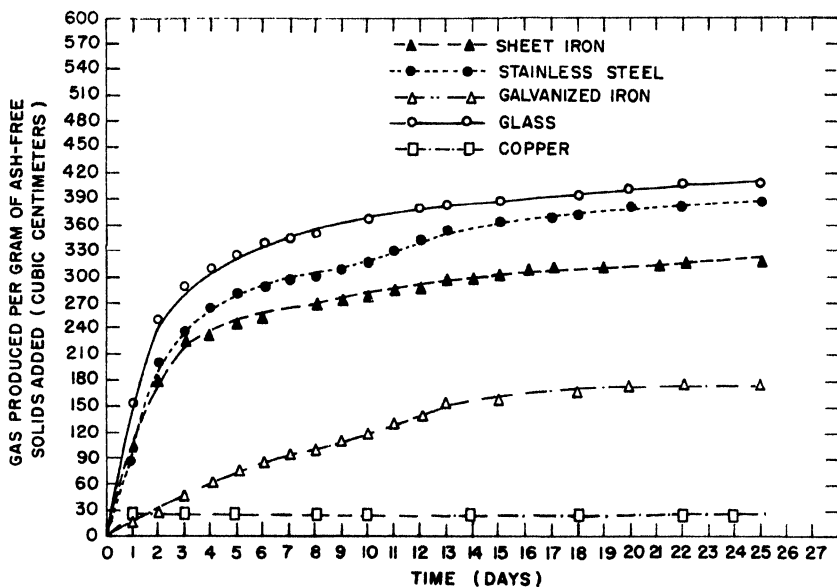
FIGURE 3.- Quantity of gas produced (cumulative) from a fermentation mixture of cornstalk flour and active methane-producing seed incubated at 50° to 55° C. in glass and various metal containers. Run 3.
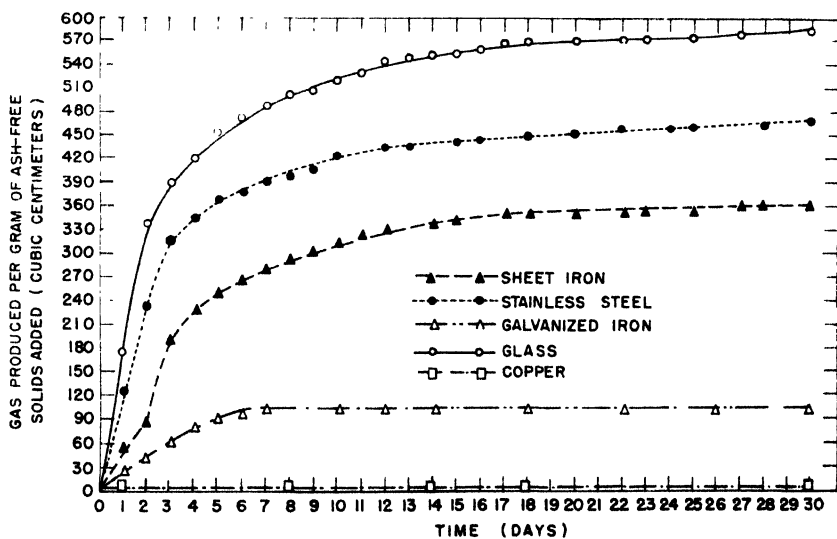
FIGURE 4.- Quantity of gas produced (cumulative) from a fermentation mixture of cornstalk flour and active methane-producing seed incubated at 50° to 55° C. in glass and various metal containers. Run 4.
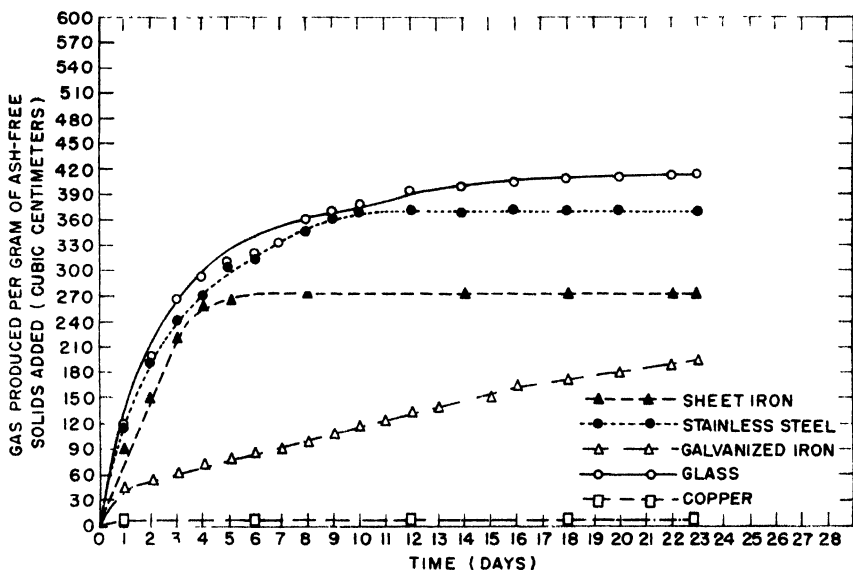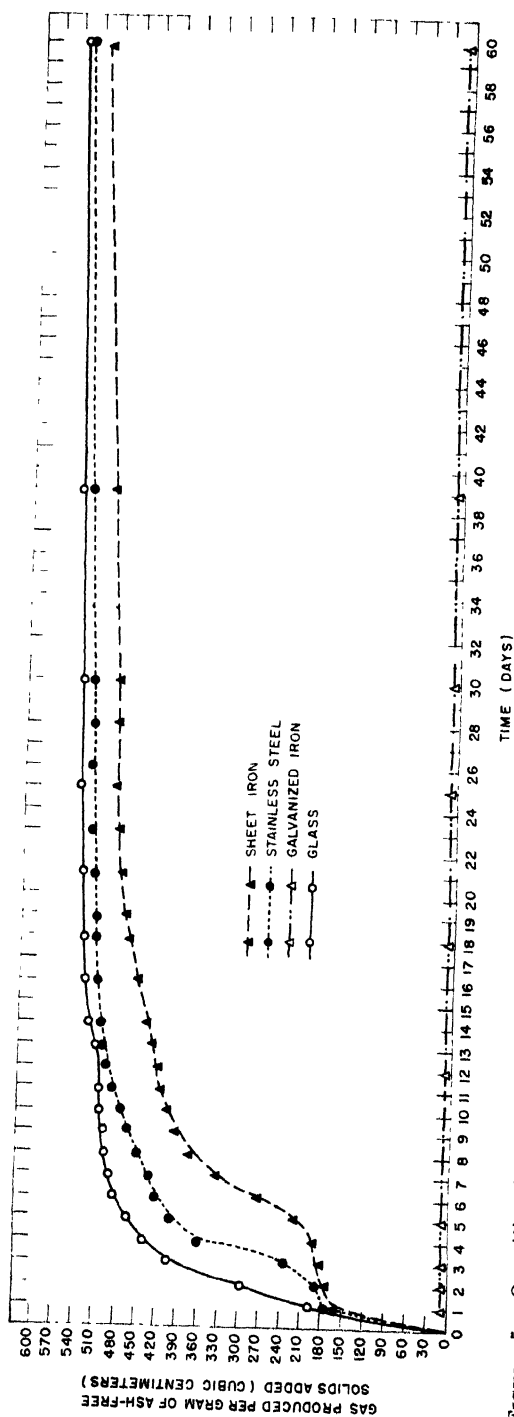
FIGURE 5.—Quantity of gas produced (cumulative) from a fermentation mixture of cornstalk flour and active methane-producing seed incubated at 50° to 55° C. in glass and various metal containers. Run 5.

TABLE 3.— *Decomposition of total and ash-free solids in cornstalk flour fermented anaerobically at 50° to 55° C. and gas produced, runs 3, 4, and 5 analyzed separately*

RUN 3 (FERMENTED FOR 25 DAYS)

Item	At start in—					After fermenting in—				
	Glass container	Galvanized-iron container	Copper container	Sheet-iron container	18-8 stainless-steel container	Glass container	Galvanized-iron container	Copper container	Sheet-iron container	18-8 stainless-steel container
Constituents										
Total solids grams	1 127 96	1 142 9	1 170 16	1 132 35	1 133 11	87 73	116 48	162 57	91 73	94.07
Ash-free solids do	1 107 22	1 121 04	1 144.26	1 109 28	1 111 50	65 22	92 15	135.47	66.29	70.75
Decomposition of cornstalk flour										
Total solids percent						63 95	41 95	12 06	64 60	62 10
Ash-free solids do						69.20	47 55	14.47	70 75	67 15
Gas produced under 30 inches of Hg at 60° F..										
Total cc						24,800	10,815	1,655	19,365	23,595
Per gram of cornstalk flour cc						394	172	26	308	375
Per gram of ash-free solids in cornstalk flour cc						408	178	27	319	389
Per gram loss of ash-free solids cc						591	374	188	451	579
Average composition of gas:										
CO₂ percent						41 1	29 4	3 1	34 4	34 2
H₂ do						2.1	5.7	6 0	1 8	3 1
CH₄ do						50 6	51 1	3 8	44 5	52.6
pH range						7 4-7 8	7 1-7 6	7 4-7 6	7 2-8 0	7 3-7 8

RUN 4 (FERMENTED FOR 30 DAYS)

Constituents										
Total solids grams	2 148 27	2 176 01	2 220 39	2 152 08	2 154 48	98 79	159 18	216.75	103.26	107.72
Ash-free solids do	2 124 40	2 150 37	2 194 42	2 125 41	2 129 82	74 74	131.89	185 62	75 27	82 33
Decomposition of cornstalk flour										
Total solids percent						78 60	26 70	5 79	77 55	74 35
Ash-free solids do						81.81	30 40	14 48	82 45	78 20
Gas produced under 30 inches of Hg at 60° F										
Total cc						35,415	6,295	230	21,935	28,260
Per gram of cornstalk flour cc						562	100	3 7	348	449
Per gram of ash-free solids in cornstalk flour cc						583	104	3 8	361	465
Per gram loss of ash-free solids cc						713	341	26	438	595
Average composition of gas										
CO₂ percent						39 8	16 9		24 4	31 0
H₂ do						0 5	0 9		1 2	1 6
CH₄ do						50 0	19 5		52 6	51 3
pH range						7 3 7 6	7 0-7 3	7.3-7 4	7 4	7 4

RUN 5 (FERMENTED FOR 60 DAYS)

Constituents										
Total solids grams	3 160 23	3 218 61		3 164 48	3 168 99	93 83	203 75		99 86	92 33
Ash-free solids do	3 134 81	3 190 04		3 135 27	3 142 24	68 05	174 46		65 68	65 79
Decomposition of cornstalk flour										
Total solids percent						105 40	23 60		102 90	121 70
Ash-free solids do						109 90	25 60		114.60	125 70
Gas produced under 30 inches of Hg at 60° F..										
Total cc						34,105	680		32,265	33,890
Per gram of cornstalk flour cc						542	11		512	538
Per gram of ash-free solids in cornstalk flour cc						562	11.2		531	558
Per gram loss of ash-free solids cc						511	44		464	443
Average composition of gas:										
CO₂ percent						37 4			27 2	29 3
H₂ do						1 6			1 7	1.5
CH₄ do						52 7			56 0	55 2
pH range						7 4-7 6	7 0-7 1		7 2-8 0	7 4-7 6

[1] From 65 gm. of cornstalk flour, 0.5 gm of urea, and bacterial seed.
[2] From 65 gm. of cornstalk flour, 1 0 of urea, and bacterial seed.
[3] From 65 gm. of cornstalk flour, 2.0 gm. of urea, and bacterial seed.

COMBINED RUNS NOS. 3, 4, AND 5

As previously mentioned, a complete analysis was made of the fermenting batches after runs 1 and 2, but not after runs 3 and 4. At the end of run 5 (after run 4 in the copper container), a complete analysis was again made. This constituted an analysis of the combined runs 3, 4, and 5. These data are presented in table 4.

TABLE 4.— *Decomposition of constituents in cornstalk flour fermented anaerobically at 50° to 55° C. and gas produced, runs 3, 4, and 5 combined* [1]

	At start in -					After fermenting for 121 days in				
Item	Glass container[2]	Galvanized-iron container[2]	Copper container[2]	Sheet-iron container[2]	18-8 stainless-steel container[2]	Glass container	Galvanized-iron container	Copper container	Sheet-iron container	18-8 stainless-steel container
Constituents										
Total solids _ grams_	[3]256 90	[3]271 84	[4]234 13	[3]261 29	[3]262 05	93.83	203.75	216 75	99 86	92 33
Ash-free solids do	[3]231 70	[3]245 52	[4]206 00	[3]233 76	[3]235 98	68 05	171 46	185 62	65 68	65 79
Pentosans _ do	66 57	70 87	61.11	67 13	67 08	3 92	43 45	51.91	3 10	2 67
Crude cellulose (ash-free) grams	96 72	104 33	83 04	98.29	97 02	11 79	76 94	67 37	10 24	10.65
Cellulose (corrected [5]) grams	58 83	63 83	54 31	59 13	58 74	10 14	52 39	48 40	8 89	9 39
Lignin___ __ ____do _	50 35	52 47	41.83	51 50	52 39	39 72	48 72	40 99	38 96	40 49
N2 in lignin___ percent						2 48	2 90	2 16	2 86	2 61
Decomposition of constituents in cornstalk flour										
Total solids___ percent _						86 35	36 10	13 82	85 45	89 60
Ash-free solids__ do __						89 75	39 01	16 78	92 25	94 25
Pentosans ____ do						101 50	44 45	22 35	103 70	104 20
Crude cellulose (ash-free) percent						95 20	30 75	26 40	98 80	96.95
Cellulose (corrected [5]) percent						92 20	21 65	16 78	95 00	93 45
Lignin_ do						34 55	14 50	4 05	40 80	38 75
Gas produced under 30 inches of Hg at 60° F Total_____ cc						94,320.	17,790	1,885	73,565	85,745
Per gram of cornstalk flour___ ___ cc						499	94	15	389	451
Per gram of ash-free solids in cornstalk flour_cc						518	98	15 6	404	471
Per gram loss of ash-free solids ___ ____cc__						576	250	93	438	504

[1] Only 4 runs were made in the copper container

[2] At the end of runs 3 and 4 a small sample (100 cc) of the fermenting mixture was removed, and determinations were made only for total solids and ash-free solids   At the end of run 5 a liter sample (as in runs 1 and 2) was used for a complete analysis. Before beginning run 3, in addition to the cornstalk flour a liter of water was also added This liter of water replaced the liter of fermenting mixture removed for a complete analysis.   The addition of the water added a dilution factor which was taken into consideration when the figures in table 4 were computed   A complete analysis of the fermenting mixture in the copper container was made at the end of run 4

[3] A total of 195 gm. of cornstalk flour and a total of 3 5 gm of urea were added to the seed for runs 3, 4, and 5

[4] A total of 130 gm. of cornstalk flour and a total of 1 5 gm. of urea were added to the seed for runs 3 and 4.

[5] Corrected for pentosans and ash.

The scoring of the containers was done on the same basis as in runs 1 and 2. With 30 as the highest possible score, the containers were rated as follows: Stainless steel, 24.5; glass, 24.0; sheet iron, 23.5; galvanized iron, 12.0; and copper, 6.0.

RATINGS BASED ON ALL RUNS

The sum of the scores for all runs, with 127 as the highest value possible, gives the following ratings: Glass, 109.0; stainless steel, 104.5; sheet iron, 90.5; galvanized iron, 58.5; and copper, 28.0. The scores are summarized in table 5.

TABLE 5. - *Summary of ratings of the various containers*

Run No	Ratings for container made of--				
	Glass	Stainless steel	Sheet iron	Galvanized iron	Copper
1	22 5	24 0	21 5	16 0	6 0
2	28 0	21 0	17 5	13 5	10 0
3	13 0	12 0	9 0	8 0	3 0
4	13 0	12 0	11 0	6 0	3 0
5	8 5	10 5	8 0	3 0	-----
Combined 3, 4, and 5 [1]	24 0	24 5	23 5	12 0	⁴6 0
Total	109 0	104 5	90 5	58 5	28 0

[1] Analysis of combined runs 3, 4, and 5 differs from that of the individual runs 3, 4, and 5 in that it includes the pentosans, cellulose, and lignin while the analyses of the individual runs do not.
[2] Does not include run 5.

QUANTITIES OF THE METALS IN THE FERMENTING MIXTURES AT THE END OF EACH RUN

The quantities of the metals present in the fermenting mixtures at the end of each run are shown in table 6.

TABLE 6.- *Quantities of metals in the fermenting solutions of cornstalk flour[1] in containers of different composition at the end of each run*

Type of container	Run No.	Quantity present at end of run			Type of container	Run No	Quantity present at end of run		
		Iron	Copper	Zinc			Iron	Copper	Zinc
		P p m	*P p m.*	*P p m*			*P p m*	*P p.m*	*P p m.*
Glass	1	314				1	629		
	2	440				2	1,915		
	3	450			Sheet iron	3	1,844		
	4	459				4	1,825		
	5	488				5	2,702		
Galvanized iron	1	419		100		1	338		
	2	509		184		2	458		
	3	818		189	Stainless steel	3	613		
	4	773		449		4	1,246		
	5	974		1,285		5	1,281		
Copper	1	312	794			At start	202	10	12 4
	2	421	894			1	136		
	3	421	996		Seed control (glass)	2	78		
	4	419	983			3	71		
						4	63		
						5	66		

[1] The cornstalk flour contained (on dry basis) per gram: 4.293 mg. of Fe, 0 01395 mg. of Cu, and 0.50 mg. of Zn.

In the interpretation of the results the following facts should be kept in mind: (1) At the beginning of runs 1, 2, and 3, a liter of water was added, thus causing a dilution factor; (2) a gram (dry basis) of the cornstalk flour contained 4.293 mg. of iron, 0.01395 mg. of copper, and 0.50 mg. of zinc (it is believed that a large part of this came from the equipment used for preparing the cornstalk flour); and (3) the seed contained some iron, copper, and zinc.

In general, there was an accumulation of iron in the fermenting masses of all containers with each succeeding run, the sheet-iron container showing the greatest increase and the copper and glass containers showing the least. At the end of run 5 (run 4 for the copper container), the quantity of iron in mixtures from the various contain-

ers was as follows: Sheet iron, 2,702 p. p. m.; stainless steel, 1,281 p. p. m.; galvanized iron, 974 p. p. m.; glass, 488 p. p. m.; and copper, 419 p. p. m. The seed control showed a decrease in iron from 202 p. p. m. at the beginning to 66 p. p. m. at the end of run 5.

Zinc determinations were made on the mixtures in the galvanized iron container at the end of each run. At the end of run 1, the zinc content was 100 p. p. m., and it gradually increased during each succeeding run to 1,285 p. p. m. at the end of run 5.

Copper determinations were made on the mixtures in the copper container at the end of each of the four runs. At the end of run 1 the copper content was 794 p. p. m., and it gradually increased to 983 p. p. m. at the end of run 4.

DISCUSSION

It would be possible to discuss the results from many angles, such as gas production, methane content, decomposition of the various constituents, and metallic content. The authors believe, however, that such an extensive discussion might confuse rather than help the reader. The primary object of the experiment was to ascertain the effect of metal containers on the continuous thermophilic anaerobic fermentation of fibrous farm wastes with repeated use of the same bacterial seed. It is believed that the criteria, including the method of rating, for judging the best type of metal container were fairly inclusive and reasonable.

The fermentations in the stainless-steel container compared favorably with those in the glass control. The quantities of gas produced in these two containers were fairly close throughout the five runs. The break-down of the various constituents, although it varied more widely than did the gas produced, was approximately of the same magnitude for the two containers. The cellulose and the pentosans were broken down the most readily; the lignin was the most resistant.

The results obtained with the sheet-iron container were rather erratic. Less gas was generally produced from the sheet-iron than from the glass container throughout the five runs. However, the relative quantities of gas produced from the sheet-iron container varied considerably from run to run, the smallest quantity being produced in run 2 and the largest in run 5. It is also interesting to note that, while less gas was produced in the sheet-iron container than in the glass, the breakdown of the various constituents was approximately of the same magnitude for both containers. Cellulose and pentosans were broken down the most readily, and lignin was the most resistant of the plant constituents.

The results obtained with the galvanized-iron and with the copper containers were rather unfavorable. Practically no gas was produced from the copper container throughout the four runs. Decreasing quantities of gas were obtained from the galvanized-iron container, and run 5 produced practically no gas. Greater quantities of the various constituents were broken down than could be accounted for by the gas produced throughout the runs in both the copper and the galvanized-iron containers.

SUMMARY

The effect of metal containers, as compared with glass, on the continuous thermophilic anaerobic fermentation of cornstalk flour by repeated use of a methane-producing seed was studied.

The stainless-steel (18–8) container was approximately as satisfactory as the glass container.

The results with the sheet-iron container were erratic and not so satisfactory as those obtained with the stainless steel.

The galvanized-iron and the copper containers were unsatisfactory.

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INTERGENERIC HYBRIDIZATION OF CEREALS AND OTHER GRASSES [1]

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INTRODUCTION

Recent investigations with cereal and other grass species have indicated the possibility of obtaining new plant types by hybridization. Such crosses should be useful in eliminating certain undesirable characteristics possessed by many grasses otherwise very satisfactory agronomically. Improved yields of forage, either as pasture or hay, earliness, winter hardiness, ability to withstand drought, and higher grain yields have been claimed for certain segregates of wide crosses. Similarly, resistance of segregates to leaf and stem rusts, smuts, and mildew has been obtained. Susceptibility to shattering and lodging has been reduced.

Transfer of the generally larger size of the caryopsis in cereals to forage species would be of great practical value in reducing hazards and difficulty incident to seeding, such as accompany the culture of many common grasses. This is particularly true in wheat regions, where farmers unaccustomed to the necessity for unusual care in sowing light and small-seeded species often fail to obtain stands. Reduction or elimination of awn development would also facilitate seeding operations. Larger seeds could be much more easily threshed and processed. Increased grain yield and elimination of harsh characters such as awns might serve to increase feeding value and palatability.

Disadvantages of intergeneric hybrid progenies might be encountered in earlier generations at least. These would include comparative sterility, a probable lack of uniformity, perhaps susceptibility to shattering, and other weaknesses.

The forage improvement program has included extensive studies of exotic and endemic grass species and the application of basic breeding methods to these in an effort to obtain superior strains. Attention has been given to the possibilities of wide crosses as a means of supplementing the basic procedure. The investigations reported herein concern intergeneric hybridization of common wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and rye (*Secale cereale* L.) with other genera, principally in the tribe Hordeae, and of common oats (*Avena sativa* L. and *A. byzantina* C. Koch), chiefly with other members of the Aveneae. Descriptions of the hybrids are not given, since work with these is still in progress and only early generations have been grown.

REVIEW OF LITERATURE

WHEAT-GRASS CROSSES

The earliest and most extensive investigations on cereal-grass crosses have been reported by Russian workers. Much of the literature is not generally available. While some of the citations refer to

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Triticum durum Desf., these are considered to be pertinent to the present discussion. In some instances statements have been found to be quite general with insufficient detail as to specific parentage of hybrids.

The first *Triticum-Agropyron* hybrids known were made by Tzitzin in 1930, according to Armstrong (*4*).[2] Tzitzin (*30*) later reported, in 1933, that *Agropyron glaucum* Desf., *A. trichophorum* Link, *A. elongatum* (Host) Beauv., and *A. junceum* (L.) Beauv. would cross with wheat. This he again reported in 1940 (*35–a*). The F_1 hybrids of wheat and *A. elongatum* were characterized by a very vigorous root system and resistance to rust, smut, and mildew. They were also resistant to cold and drought. A large percentage of the second-generation plants were perennial. Some of these had luxuriant vegetative growth and were said to be promising as forage plants. In 1935 Tzitzin (*31*) confirmed the previous claims as to drought resistance and productivity of *Triticum-Agropyron* hybrids and indicated the yielding capacity of perennial forms to be increasing from year to year. F_2 plants varied greatly in fertility (*33*). In 1936 Tzitzin (*32*) stated that 60 to 70 percent of the F_4 generation were perennials and that constant forms having the wheat type of grain and the perennial habit had been isolated. Some of these were very early and many were more drought-resistant than wheat. In the same report Tzitzin announced the obtaining of hybrids between wheat and *Agropyron repens* (L.) Beauv. or a closely related form.

Vakar (*36*) attempted to cross *Triticum vulgare* (*T. aestivum*) and other wheat species with *Agropyron repens*, *A. tenerum* Vasey (*A. trachycaulum* (Link) Malte), *A. glaucum*, and *A. elongatum*. Hybrids were obtained only with *A. elongatum*, and the F_1 plants were completely sterile.

Veruschkine (*38*) stated that the cross *Triticum vulgare* × *Agropyron elongatum* was the most fertile of the wheat-*Agropyron* hybrids. Combinations were also reported to have been obtained between wheat and *A. elongatiforme* and *A. junceum*. Veruschkine and Shechurdine (*40*) reported that the hybrid seeds of *T. vulgare* × *A. elongatum* possessed apparently normal embryos and abortive endosperms, while seeds from *T. durum* × *A. elongatum* appeared to be normal.

Tzitzin (*34*) obtained perennial forms of the wheat type from *Triticum vulgare* var. *lutescens* × *Agropyron glaucum*. Armstrong (*4*) made crosses of two strains of *A. elongatum* and *A. glaucum* with three hexaploid and two tetraploid wheats. Attempts to combine *A. desertorum*, *A. dasystachyum* (Hook.) Scribn., *A. caninum* (L.) Beauv., *A. imbricatum* (M. B.) Roem. and Schult., *A. repens*, *A. cristatum* (L.) Gaertn. (commercial and Fairway strains), *A. obtusiusculum* Lange (*A. intermedium* (Host) Beauv.), and *A. richardsonii* Schrad. (*A. subsecundum* (Link) Hitchc.) with wheats were unsuccessful: *A. glaucum* combined more frequently with the tetraploids, while *A. elongatum* gave best results with hexaploid wheats.

Veruschkine and Shechurdine (*40*) crossed a pure line of *Triticum vulgare* var. *lutescens* with *Agropyron intermedium*. The latter species was found to cross readily with spring and winter wheats. Hybrids between *T. vulgare* and *A. intermedium* were sterile, while those having *T. durum* as the female parent were usually so. The seeds of the former cross possessed clearly developed embryos and usually aborted endosperms, while those of the latter appeared to be normal. Back-

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[2] Italic numbers in parentheses refer to Literature Cited, p. 46.

crosses of *T. vulgare* × *A. intermedium* hybrids to wheat resulted in a low seed set. Raw (*27*) reported studies of hybrids between *T. vulgare* and *A. intermedium*.

Khižnjak (*13*) reported that *Triticum vulgare* × *Agropyron intermedium* hybrids formed unreduced egg cells previous to the production of sesquidiploids (derivatives from backcrossing) and triple hybrids. The same worker (*14*) referred to second and third generation hybrids of the cross *T. durum* × *A. intermedium*, though these were presumably the results of various backcrossing combinations. Some hybrids of 28-chromosome wheats with *A. intermedium* were self-fertile owing to the production of amphidiploid combinations. Khižnjak (*12, 13*) reported a triple hybrid from *T. durum* × *A. intermedium* pollinated by *T. vulgare*.

Vakar (*37*) summarized the results of cytological studies of F_1 to F_6 of *Triticum vulgare* × *Agropyron intermedium* (sterile) × *T. vulgare*, indicating that advanced generations had been obtained. Veruschkine (*39*) stated that some forms of *A. cristatum* cross with *T. timopheevizhuk*.

Longley and Sando (*19*) obtained crosses between Fulhio and Dawson × Kanred, varieties of *Triticum aestivum*, and *Secale montanum* Guss. In 1936 Deržavin (*7*) reported that two wheat varieties had been crossed with *Secale montanum*, and a fertile, perennial, hard wheat was obtained from the progeny. Guard (*9*) found that *T. aestivum* var. Chinese crossed readily with *S. montanum*.

Johnson (*10*) studied the compatibility of 27 forms of *Triticum* crossed with 18 species of *Agropyron*. The former included 17 varieties of *T. vulgare*, two wheat-rye hybrids, *T. durum*, *T. dicoccum* Schrank, two strains of *T. turgidum* L., *T. polonicum*, *T. persicum*, *T. timopheevi*, and *T. monococcum*. The *Agropyron* species used were *A. caninum*, *A. cristatum* (two strains), *A. dasystachyum*, *A desertorum*, *A. elongatum* (six strains), *A. glaucum*, *A. griffithsii*, *A. imbricatum*, *A. intermedium*, *A. junceum*, *A. obtusiusculum*, *A. pauciflorum*, *A. repens*, *A. richardsonii*, *A. sibiricum* (Willd.) Beauv., *A. smithii* Rydb., and *A. spicatum* (Pursh.) Scribn. and Smith. Johnson reported that *Agropyron elongatum* and *A. glaucum* were the only species crossing readily with *Triticum*, and these crossed only with *T. vulgare*, *T. durum*, and *T. dicoccum*. One plant of *T. persicum* × *A. junceum* was obtained. The seeds set ranged from 34 percent, in a cross between *A. glaucum* and one variety of *T. vulgare*, to 0, in several combinations of generally compatible crosses. The F_1 hybrids with *A. glaucum* were completely sterile, while a fair proportion were moderately fertile in the *A. elongatum* cross. Many germless seeds developed from certain combinations of parents. Germless seeds and stimulated ovaries were obtained from the use of *A. junceum*, *A. desertorum*, *A. imbricatum*, *A. sibiricum*, *A. intermedium*, and *A. dasystachyum*.

Crosses between spring and winter wheats and *Agropyron glaucum* and *A. trichophorum* were made by Nemlienko (*21*). More seeds set when these species were crossed with *Triticum durum* than with *T. vulgare*, and these were more viable in the first-named cross.

White (*42*) reported successful hybridization of nine varieties of *Triticum vulgare* with *Agropyron elongatum*. In crosses between the same wheat varieties and *A. glaucum*, seven set seed. Differences in seed setting were noted among the wheat parents, and hybrid seeds

from pollination by *A. glaucum* were only about one-third as numerous as when *A. elongatum* was used. Seeds were also better developed in the latter instance. Hybrids between *Triticum* species and *A. tricho-phorum* were reported.

In the United States, W. J. Sando, of the Division of Cereal Crops and Diseases, has worked extensively with wheat-*Agropyron elongatum* crosses, many hybrid combinations having been obtained.[3]

Michels[4] described a perennial hybrid between Mosida (*Triticum aestivum*) and *Elymus condensatus* Fresl., which was released in 1938 by the Idaho Agricultural Experiment Station. Poddubnaja-Arnoldi (*25*) obtained no normally developed seeds from crosses between *Triticum* and *Elymus* species, though in some instances embryos and endosperms began to be differentiated.

RYE-GRASS CROSSES

Crosses between *Secale cereale*×*S. montanum* were described by Kostoff (*15*) in 1931. The same hybrid was reported by Antonoff (*2*) and Deržavin (*7*) in 1936. Hybrids of *S. cereale* and other *Secale* species were discussed by Kostoff (*16*) in 1937. The F_1 of *S. cereale*× *S. montanum* was somewhat fertile, while F_1 plants of other combinations were highly fertile. Duka (*8*) in 1938 discussed work with Petkus rye crossed with *S. montanum* in 1930. Studies were made of F_2 to F_6 generations. In 1938 Rezničuk (*28*) reported making the same cross.

E. G. Schafer, of the Washington Agricultural Experiment Station, crossed *Secale cereale* with *S. montanum* in 1928. Several generations have been grown and selections have been made, primarily for grain but also for forage types. W. J. Sando has also made and studied this cross.[5]

Scattered reports of crosses of *Secale cereale* with other grass genera are available. Veruschkine and Shechurdine (*40*) obtained a hybrid seed set of 30.47 percent crossing *Secale cereale* × *Agropyron inter-medium*. Crasniuk (*6*) in 1935 reported a hybrid between a fifth-generation inbred plant of a Russian rye, Eliseevskaja, and *Agropyron cristatum* (L.) P. B. var. *imbricatum* (M. B.) Moiss. This variety sometimes has been regarded as an independent species, *A. imbrica-tum*. One plant was obtained which proved to be a perennial. Pollen was almost completely sterile, and backcrosses were unsuccessful. Aldanov (*1*) and Veruschkine (*39*) likewise found that *S. cereale* and *A. cristatum* could be crossed successfully. In 1937 Ljubimova (*18*) reported the hybrid *S. cereale* × *A. glaucum* var. *genuinum*, which proved to be completely sterile. Tsitsin (*29*) also indicated that "rye-couch grass hybrids" had been obtained.

BARLEY AND OAT CROSSES WITH GRASSES

Very few workers have investigated hybridization of barley or oats with grasses. Malloch (*20*) pollinated *Hordeum vulgare pallidum* with *H. nodosum* L. but obtained no hybrid seeds. In crosses of *H. vulgare trifurcatum* × *H. "murianum"* (probably *H. murinum* L.), two viable seeds developed which resembled the maternal variety. After a retarded germination, seedlings died when 2 to 4 inches tall. Kuckuck (*17*) attempted to hybridize *H. sativum* and *H. bulbosum* L. With the

[3] Unpublished work.
[4] MICHELS. C A. WHEAT-WILD RYE HYBRID GRASS. Idaho Agr. Expt. Sta. Leaflet 40, 4 pp. 1940 [Mimeographed.]
[5] Unpublished work.

latter variety as the female parent no seeds were obtained in 1,084 florets pollinated. In the reciprocal cross 4,578 florets of several varieties were pollinated. As a result 349 seeds were obtained, though these were only partially developed and usually were embryoless. One hybrid plant was obtained which upon examination proved to have 21 chromosomes in the somatic tissue. Rezničuk (*28*) attempted to cross *H. bulbosum* and *Elymus* species with cultivated barley but obtained no seeds. Quincke (*26*) has reported the crosses *H. jubatum* L. × *H. vulgare* and *H. nodosum* × *H. vulgare*.

No literature describing attempts to hybridize cultivated oats (*Avena sativa* or *A. byzantina*) with grasses of other genera has been seen by the author, though Johnson and McLennan (*11*) attempted unsuccessfully to cross the cultivated oat species with perennial types.

MATERIALS AND METHODS

The cereal parent was used as the female in all crossing combinations. Emasculation was accomplished by the use of forceps in the usual manner for small grains. Wheat spikes were reduced to 8 to 10 spikelets with 2 florets per spikelet. Barley heads were trimmed to 8 to 12 spikelets. Eight to 12 spikelets, each with 2 florets, were left on the rye rachis. Oat panicles were cut to 8 to 12 primary florets. Rye and barley florets were cut horizontally at about three-fifths of the length from the base to facilitate emasculation and pollination.

Inflorescences were covered with tissue-paper wrappings or parchment bags prior to pollination, which was accomplished 3 to 5 days after emasculation. In the case of rye it was done somewhat later. Pollinated heads were re-covered and left to mature.

All of the seeds obtained in crossing operations were grown whether or not they appeared to be typical of the female parent. In some instances, particularly in rye, badly shrunken seeds produced normal plants.

In making hybrids, coincident production of receptive stigmas and fertile pollen is necessary. This was managed in the present studies by using several cereal varieties of each species, seeded at different dates. Subsequently the choice of a cereal parent was dependent only upon its being in the proper state of maturity when grass pollen was available.

Since the cereal varieties used in crossing are not indicated in the tabular summary, except for actual hybrids, they will be named to indicate the types utilized. Most of these bear identification numbers of the Division of Cereal Crops and Diseases of the Bureau of Plant Industry. The origin of others will be indicated briefly. All lots were previously grown at Pullman, Wash., by the Division of Cereal Crops and Diseases and the Washington Experiment Station.

Wheat varieties, in approximate order of frequency of use, were White Odessa-Hohenheimer, White Odessa-Heils Dickkopf, Ridit, Turkey-Florence, Regenerated Defiance, Federation, Marquis, Rex, Red Russian, Red May, Kinney, Redhart, and White Winter. White Odessa-Hohenheimer and White Odessa-Heils Dickkopf are strains derived by the Washington station from the corresponding crosses. Hybrid strains of the varieties Hohenheimer and Heils Dickkopf [6] are soft, white, late-winter wheats. Their extensive use was due primarily to their late maturity, coincident with flowering of grasses. Turkey-
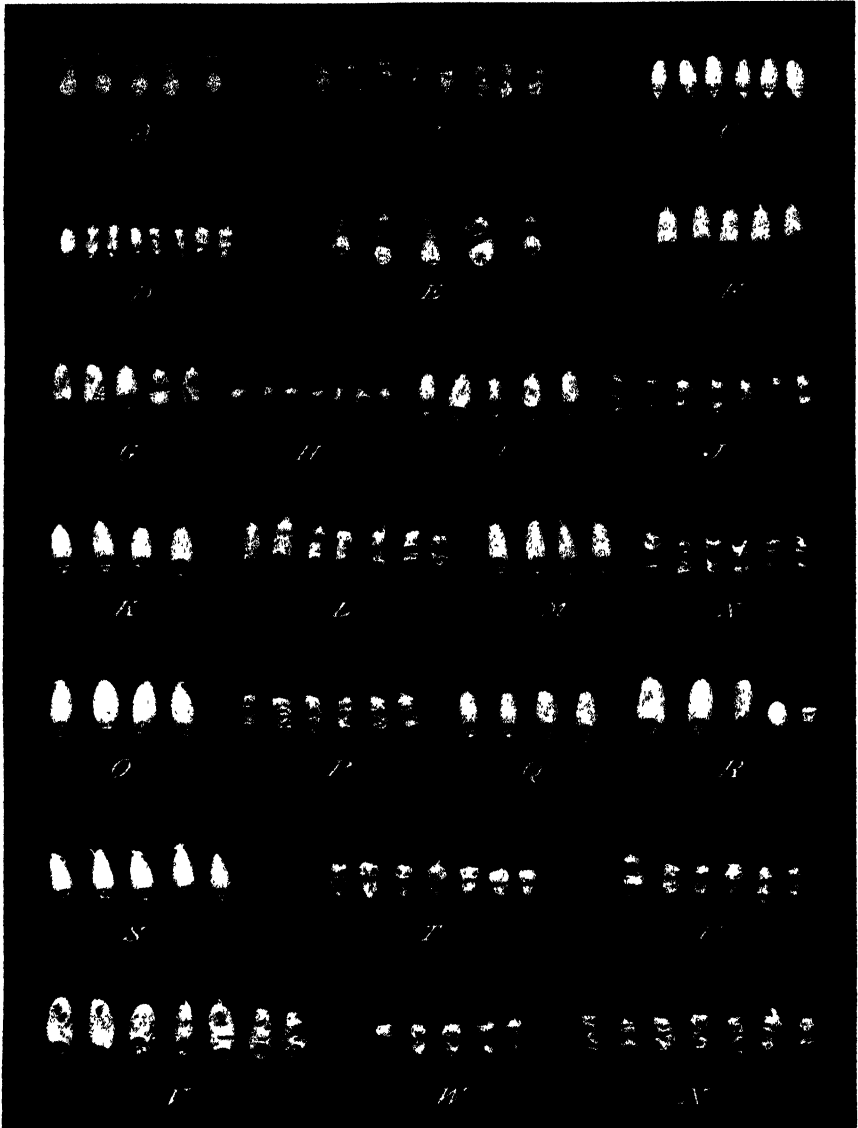
Seeds of wheat varieties and of wheat varieties crossed with various grass species.

A, White Odessa-Heils Dickkopf.   *B*, White Odessa-Heils Dickkopf × *Agro-pyron intermedium*.   *C*, White Odessa-Heils Dickkopf.   *D*, White Odessa-Heils Dickkopf × *Agropyron trichophorum*.   *E*, White Odessa-Hohenheimer; result of pollination with grass pollen; not a hybrid.   *F*, Kinney.   *G*, Kinney × *Agro-pyron elongatum*.   *H*, Unfertilized, pollinated White Winter wheat.   *I*, Marquis. *J*, Marquis × *Agropyron repens*.   *K*, White Winter.   *L*, White Winter × *Agro-pyron intermedium*.   *M*, White Odessa-Heils Dickkopf.   *N*, White Odessa-Heils Dickkopf × *Agropyron intermedium*.   *O*, White Odessa-Hohenheimer.   *P*, White Odessa-Hohenheimer × *Agropyron intermedium*.   *Q*, Regenerated De-fiance.   *R*, Regenerated Defiance × *Agropyron intermedium*.   *S*, Red Rus-sian.   *T*, Red Russian × *Agropyron intermedium*.   *U*, Marquis × *Agropyron trichophorum*.   *V*, White Odessa-Hohenheimer × *Elymus condensatus*.   *W*, White Odessa-Heils Dickkopf × *Agropyron sibiricum*.   *X*, White Odessa-Heils Dickkopf × *Agropyron intermedium*.

Florence is a hard, white-grained, late-winter selection from the indicated cross.

Rye varieties included Rosen, Dakold, and a local spring strain known as Prolific. The varieties Beldi Giant, Winter Club, Orel, Tennessee Winter Smooth Awn, Tennessee Winter, Nobarb, and Eureka were used in barley-grass crosses. Markton, Gray Winter, and Red Rustproof were the oat varieties utilized in the few attempts made to obtain oat-grass crosses.

The grass strains used as male parents were pedigreed lots from native collections, foreign-plant introductions, and commercial sources. Chromosome numbers determined for these species by other workers are listed in table 1, as a descriptive feature of interest.[7] Since strains within species may vary widely in chromosome number, those given may be considered as suggestive only of the possible counts in the grass strains actually used, whose numbers have not been determined.

Investigations were begun in 1937 and continued, the present summary including the seasons from 1937 to 1939.

TABLE 1.—*Grass species used in crosses with chromosome numbers as reported by other investigators*

Species	Chromosome number (2n)	Authority and date	Reference
Agropyron caninum (L) Beauv	28	Peto, 1930 [1]	(41)
Agropyron cihare (Trin.) Franch	28	Nakajima, 1936	(41)
Agropyron cristatum (L) Gaertn	14	Peto, 1930 [1]	(24)
Do	28	do [1]	(41)
Do	42	Araratian, 1938	(3)
Agropyron dasystachyum (Hook) Scribn	28	Peto, 1930	(41)
Agropyron elongatum (Host) Beauv	70	do.[1]	(41)
Agropyron inerme (Scribn and Smith) Rydb			
Agropyron intermedium (Host) Beauv	42	Peto, 1930 [1]	(41)
Agropyron pungens (Pers) Roem and Schult	42	do [1]	(41)
Agropyron repens (L) Beauv	42	Stolze 1925 [1]	(41)
Agropyron semicostatum (Steud) Nees	28	Nielsen and Humphrey, 1937 [1]	(23)
Agropyron sibiricum (Willd.) Beauv	28	Avdulow, 1928	(41)
Agropyron smithii Rydb	56	Peto, 1930 [1]	(41)
Agropyron spicatum (Pursh) Scribn. and Smith	14	do [1]	(41)
Agropyron trachycaulum (Link) Malte	28	do.[1]	(41)
Agropyron trichophorum (Link) Richt	42	Araratian, 1938	(3)
Arrhenatherum elatius (L) Mert and Koch	28	Aase and Powers, 1926 [1]	(41)
Bromus carinatus Hook and Arn	56	Stählin, 1929	(41)
Bromus inermis Leyss	42	do	(41)
Do	56	Avdulow, 1928	(41)
Do	56-70	Nielsen, 1939	(22)
Dactylis glomerata L	28	Davies, 1927 [1]	(41)
Elymus ambiguus Vasey and Scribn. (var *strigosus*	28	Nielsen and Humphrey, 1937	(23)
Elymus angustus Trin			
Elymus arenarius L.*			
Elymus canadensis L	28	Avdulow, 1928 [1]	(41)
Do	42	Nielsen and Humphrey, 1937	(23)
Elymus condensatus Presl	28	do	(23)
Elymus giganteus Vahl	28	Avdulow, 1931	(41)
Elymus glaucus Buckl			
Elymus junceus Fisch			
Elymus triticoides Buckl			
Festuca elatior L	14	Stolze, 1925	(5)
Festuca idahoensis Elmer			
Hordeum bremsubulatum (Trin) Link			
Hordeum bulbosum L	28	Stählin, 1929 [1]	(41)
Hordeum jubatum L	14	Tanji, 1925	(41)
Do	28	Aase and Powers, 1926 [1]	(41)
Hordeum nodosum L	14	Tanji, 1925	(41)
Do	42	Griffee, 1927	(41)
Koeleria cristata (L.) Pers	70	Avdulow, 1931	(41)
Lolium perenne L	14	Evans, 1926 [1]	(41)
Secale montanum Guss	14	Stolze, 1925 [1]	(5)

[1] Substantiated by other investigators.

[7] Since this manuscript was prepared, Stebbins and Love (29) have reported 2n numbers to be *Elymus glaucus* 28, *E. triticoides* 42, and *Festuca idahoensis* 28. Counts for several other species listed in table 1 were verified. The 2n number for *Koeleria cristata* was found to be 28.

RESULTS

In the discussion of results a description of any hybrids obtained will be omitted, since work with these is being continued. It may be stated, however, that F_1 plants have proved to be completely sterile in all crosses studied except that of *Secale cereale* $\times$ *S. montanum*.

WHEAT-GRASS CROSSES

Data summarizing the results with hybrids having wheat as the female parent are listed in table 2. Columns 3 and 4 indicate the number of different wheat varieties and grass strains used with each grass species. The numbers of seeds indicate only those seeds that appeared to be hybrid or later produced hybrid plants. Occasional selfed seeds were found, but these were eliminated from the summary. Emasculated unpollinated checks set less than 0.5 percent of seeds. It appeared that during the time the covering was removed for pollination stray pollen became effective.

Many seeds, possibly of hybrid origin, failed to germinate. Others, undoubtedly hybrid, also failed to grow. In plate 1, *E*, *V*, and *W*, deformed seeds that failed to grow or were not hybrid are shown. Some seeds appeared to be without embryos, e. g., the two on the right in plate 1, *E*. Normal grains of the same variety are shown in plate 1, *A*, and pollinated, unfertilized ovaries may be seen in plate 1, *H*.

TABLE 2.— *Summary of results of artificial pollination in attempted hybridization of common wheat (Triticum aestivum) with other grass species*

Grass species	Period of study	Wheat varieties	Grass strains	Total florets	Hybrid seeds	Hybrid plants
	Years	Number	Number	Number	Number	Number
Agropyron caninum	1	2	2	534	0	0
Agropyron ciliare	1	2	1	81	0	0
Agropyron cristatum	3	4	4	882	1	1
Agropyron dasystachyum	3	3	1	80	3	0
Agropyron elongatum	1	2	1	332	17	11
Agropyron inerme	3	4	2	701	0	0
Agropyron intermedium	1	5	2	2,408	363	20
Agropyron pungens	1	2	1	426	0	0
Agropyron repens	2	1	3	720	0	0
Agropyron semicostatum	1	1	1	140	0	0
Agropyron sibiricum	1	5	1	578	0	0
Agropyron smithii	2	3	1	356	0	0
Agropyron spicatum	1	3	1	716	0	0
Agropyron trachycaulum	2	4	2	710	0	0
Agropyron trichophorum	3	7	1	1,128	76	16
Elymus angustus	1	1	1	416	0	0
Elymus canadensis	2	2	4	702	0	0
Elymus condensatus	3	5	4	1,619	0	0
Elymus giganteus	1	2	2	120	0	0
Elymus glaucus	1	2	2	134	0	0
Elymus junceus	1	1	1	18	0	0
Elymus triticoides	2	2	1	785	0	0
Festuca elatior	2	4	2	452	0	0
Festucaidahoensis	1	1	1	74	0	0
Lolium perenne	1	1	1	40	0	0
Secale montanum	2	2	1	610	0	0

One dwarfed seedling was derived from a cross of Rex $\times$ *Agropyron cristatum*. This plant produced five leaves before premature death. Since numerous segregating progenies of crosses between *Triticum aestivum* and *A. elongatum* have been available through the work of W. J. Sando, attempts to obtain new local combinations of this cross have not been extensive. Eleven plants from 17 hybrid seeds resulted from pollination of 286 florets of Kinney $\times$ *Agropyron elongatum*.

The nature of seeds producing hybrid plants is shown in plate 1, *G*, and the female parent in plate 1, *F*.

During the period of investigation, combinations of one wheat variety with three strains of *Agropyron repens* have been attempted. The strains of *A. repens* were used only with White Odessa-Hohenheimer, and all failed to cross with this wheat.

Fourteen hybrid plants were obtained from the cross of White Odessa-Heils Dickkopf × *Agropyron intermedium*. Hybrid seeds are shown in plate 1, *B* and *X*. Similar hybrid seeds were produced by crossing White Odessa-Hohenheimer, Red Russian, White Winter, and White Odessa-Heils Dickkopf with a shorter, finer form of *A. intermedium* resembling *A. repens*. Five seeds produced with Regenerated Defiance as a parent are shown in plate 1, *R*, plate 1, *Q*, being the normal seeds of Regenerated Defiance. The two small seeds at the right were peculiar and unlike each other or the female parent. Neither of these seeds produced hybrid plants. The three other seeds proved to be the result of selfing. Though 260 florets of Marquis were used no seeds were obtained. The percentages of shrunken seed set by the respective wheat varieties were White Odessa-Hohenheimer, 34; White Odessa-Heils Dickkopf, 18; Red Russian, 12; White Winter, 4; Marquis, 0; and Regenerated Defiance, 0. Plate 1, *A* to *T*, inclusive, illustrates the types of seeds obtained from the crosses, as compared with those of the female parents.

Six plants were obtained from wheat × *Agropyron intermedium* (short form) crosses, all coming from White Odessa-Hohenheimer used as the female parent. Incidentally this cross gave the highest percentage of seed set, as previously indicated. Seeds that produced plants were classified as either "badly shrunken" or "very badly shrunken."

Red Russian, Ridit, Regenerated Defiance, Marquis, White Odessa-Heils Dickkopf, White Odessa-Hohenheimer, and Federation, the latter 2 in a very limited way, were used in crosses with *Agropyron trichophorum*. In 1,128 florets of 7 wheat varieties, 76 seeds were obtained; 16 of these produced plants. Seeds of this cross, with the female parents, are shown in figure 1, *C*, *D*, *I*, and *U*. White Odessa-Heils Dickkopf was the most successful parent, though use of Red Russian and Marquis also resulted in hybrid seeds. Regenerated Defiance and Ridit, though more extensively used than other varieties, did not function successfully as parents.

All of the seeds obtained in crossing operations were grown whether or not they appeared to be typical of the female parent. In some instances, particularly in rye, badly shrunken seeds produced normal plants.

No crosses other than those previously discussed and indicated in table 1 were obtained from pollinations of wheat varieties.

RYE-GRASS CROSSES

Summary data indicating results of attempted crosses with rye as the female parent are given in table 3. In all, 19 species of grass comprising 27 strains were used with Rosen, Dakold, and Prolific ryes. Many seeds were obtained, most of which proved to be normal rye when resulting plants were observed.
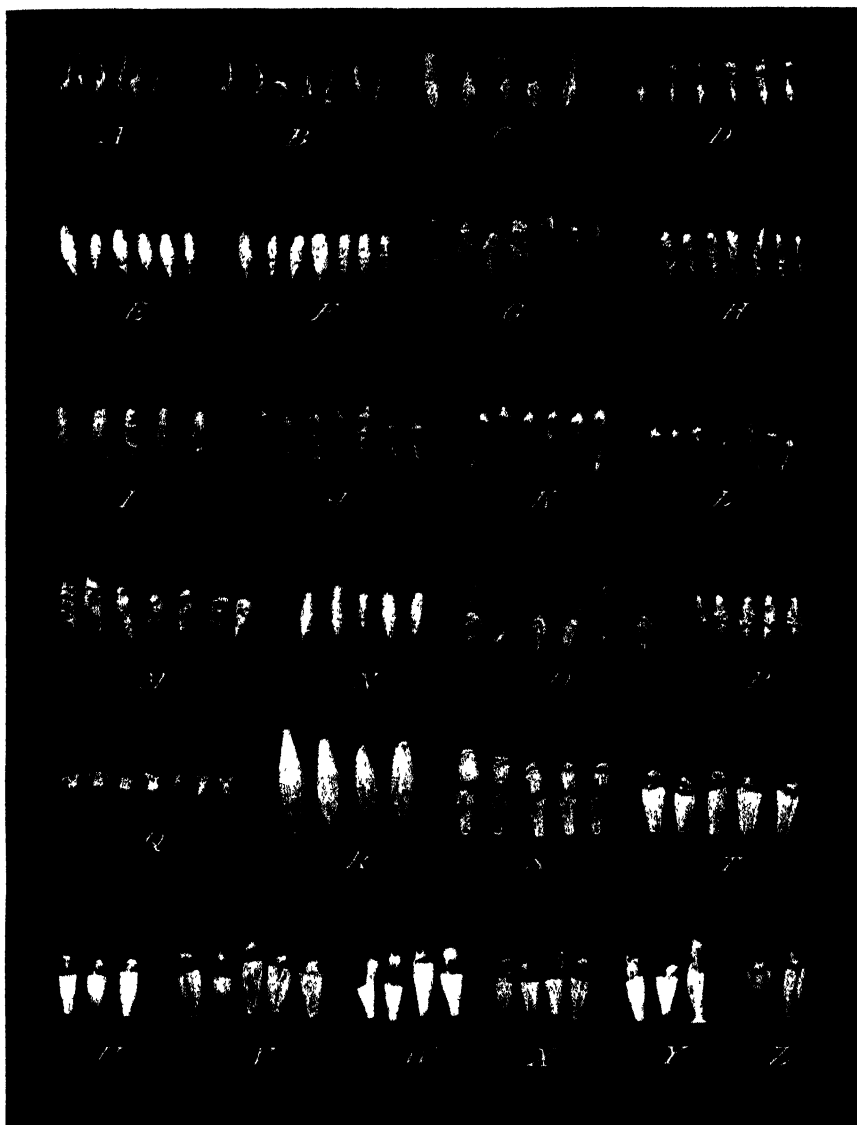
Seeds of cereal varieties and of cereal varieties crossed with various grass species.

A-P, Rye varieties and crosses: *A*, Rosen rye artificially pollinated, unfertilized; *B*, Prolific rye artificially pollinated, unfertilized; *C*, Prolific rye; *D*, Prolific rye × *Agropyron repens*; *E*, Dakold; *F*, Dakold × *Secale montanum*; *G*, Prolific rye × *Agropyron sibiricum*; *H*, Prolific Rye × *Secale montanum*; *I*, Prolific rye; *J*, Prolific rye × *Agropyron trichophorum*; *K*, Secale montanum; *L*, Prolific rye × *Secale montanum*; *M*, Rosen × *Agropyron trachycaulum*; *N*, Prolific rye; *O*, Prolific rye × *Elymus glaucus*; *P*, Prolific rye × *Elymus condensatus*. *Q-Z*, Barley varieties and crosses: *O*, Beldi Giant barley, artificially pollinated, unfertilized; *R*, Beldi Giant barley; *S*, Beldi Giant barley, pollinated, fertilized; *T*, Beldi Giant barley × *Secale montanum*; *U*, Tennessee Winter × *Hordeum bulbosum*; *V*, Orel × *Hordeum bulbosum*; *W*, White Winter × *Agropyron trichophorum*; *X*, Nobarb × *Agropyron cristatum*; *Y*, White Winter × *Secale montanum*; *Z*, White Winter × *Hordeum nodosum*.

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TABLE 3.—*Summary of results of artificial pollination in attempted hybridization of common rye (Secale cereale) with other grass species*

Grass species	Period of study	Rye varieties	Grass strains	Total florets	Hybrid seeds	Hybrid plants
	Years	*Number*	*Number*	*Number*	*Number*	*Number*
Agropyron cristatum	2	3	3	942	0	0
Agropyron dasystachyum	1	1	1	60	0	0
Agropyron pungens	1	1	1	24	0	0
Agropyron repens	1	1	1	220	43	16
Agropyron sibiricum	2	1	1	1,230	1	1
Agropyron smithii	2	2	1	486	0	0
Agropyron spicatum	2	3	1	1,486	0	0
Agropyron trachycaulum	1	2	2	444	0	0
Agropyron trichophorum	2	3	1	1,434	197	17
Bromus inermis	1	1	1	130	0	0
Elymus ambiguus	1	1	1	124	0	0
Elymus canadensis	2	1	4	1,050	0	0
Elymus condensatus	2	3	2	1,058	0	0
Elymus giganteus	1	1	1	552	0	0
Elymus glaucus	2	1	1	266	0	0
Elymus junceus	1	2	1	106	0	0
Elymus triticoides	1	1	1	602	0	0
Hordeum bulbosum	2	2	1	160	0	0
Secale montanum	1	1	1	664	207	175

Sixteen plants were obtained from 43 seeds of the cross Prolific rye × *Agropyron repens*. Hybrid seeds and those of the rye parent are shown in plate 2, *C* and *D*. Plate 2, *A* and *B*, may be used to compare seed development with ovaries of pollinated, unfertilized Rosen and Prolific ryes. While the hybrid seeds were badly shrunken, a good percentage produced plants. As indicated in table 3, attempts were not made to cross *A. repens* with Rosen and Dakold.

One plant was obtained from the cross Prolific rye × *Agropyron sibiricum*, 1,230 florets being manipulated. Though many seeds were produced, most of these were not crosses. The seed from which the plant was derived was badly shrunken, as shown in plate 2, *G*.

Pollination of 1,064 florets of Prolific rye with *Agropyron trichophorum* resulted in seeds producing 17 plants of hybrid nature. Though 197 hybrid seeds were listed as obtained (table 3), many of these were shrunken and yet were not hybrid. Actual germination of hybrid seeds is, therefore, problematical. Seeds of this cross are shown in plate 2, *J*, the Prolific rye parent being indicated in plate 2, *I*. Though 278 florets of Rosen and 92 of Dakold were pollinated with this species, no hybrid plants were obtained.

Crosses of *Secale cereale* × *S. montanum* were obtained with relative ease. The percentages of success in using Rosen and Dakold were similar, with Prolific rye somewhat lower but perhaps not significantly so. Hybrid seeds of this cross are shown in plate 2, *F, H,* and *L,* for Dakold and Prolific rye derivatives. Those from crosses with Rosen were similar in appearance. A very high percentage of the seeds of this cross were viable, and 175 hybrid plants were obtained. The common rye varieties were standard lots locally grown previous to use as parents. *S. montanum* was obtained from stray plants growing on the Washington Experiment Station farm. The nature of the seeds of this species is shown in plate 2, *K*.

Though other crosses of ryes with grasses were attempted, none was successful. Many seeds obtained from attempted control of pollination proved to be due to selfing or to accidental crossing when the spikes were uncovered for pollination. The extent of development of the endosperm in such caryopses is indicated in plate 2, *M, O,* and *P*.

Examination of pollen of the supposed F_1 plants was resorted to in some rye crosses to aid in determining whether plants resulting from crossing were actual hybrids. It was first supposed, however, that the rye parent plants would exhibit a fairly high, reasonably uniform percentage of fertile pollen. In 56 plants of Prolific rye from greenhouse-grown cultures, of which anthers were examined, none showed entirely normal pollen. In some anthers pollen had aborted completely. Many plants showed 20 to 50 percent of pollen aborted. Examination of the contents of 3 anthers from 1 floret and several florets per spike indicated wide differences, not to be considered as due to occasional aborted or partially aborted anthers in otherwise normal florets. Pollen examination as a preliminary method of determining hybrid origin was found not to be helpful in this material, since the plants examined were not hybrids, as was later proved.

BARLEY-GRASS CROSSES

Data concerning attempted hybridization of common barley with grasses are shown in table 4. No hybrid seeds sufficiently developed to produce plants were obtained. Development of the ovary was stimulated by application of pollen from certain species, principally *Hordeum bulbosum*. In plate 2, *Q*, are shown ovaries of Beldi Giant pollinated but unfertilized. Plate 2, *R* and *S*, shows normal Beldi Giant and kernels as developed after clipping of the lemma in emasculation. Plate 2, *T*, indicates the degree of development of the caryopsis following crossing with *Secale montanum*. Similar development is shown for crosses of Tennessee Winter×*Hordeum bulbosum* (pl. 2, *U*), and Orel×*Hordeum bulbosum* (pl. 2, *V*), though one seed of the latter is further developed and proved to be due to selfing. Plate 2, *W* to *Z*, indicates the development of the caryopsis when several other grass parents were utilized for pollen. Some of the better developed seeds grew, these proving to be selfs. It is evident in comparing plate 2, *T* to *Z*, with plate 2, *Q*, that partial development occurred in the former.

TABLE 4.--*Summary of results of artificial pollination in attempted hybridization of common barley (Hordeum vulgare) with other grass species*

Grass species	Period of study	Barley varieties	Grass strains	Total florets	Hybrid seeds	Hybrid plants
	Years	*Number*	*Number*	*Number*	*Number*	*Number*
Agropyron cristatum	3	3	4	379	0	0
Agropyron intermedium	1	1	1	143	0	0
Agropyron repens	1	3	1	127	0	0
Agropyron smithii	2	2	1	431	0	0
Agropyron trichophorum	1	1	1	67	0	0
Bromus carinatus	1	1	1	30	0	0
Bromus inermis	1	2	2	183	0	0
Elymus ambiguus	1	1	1	94	0	0
Elymus angustus	1	1	1	91	0	0
Elymus arenarius	1	1	1	57	0	0
Elymus canadensis	1	1	2	131	0	0
Elymus condensatus	2	2	3	185	0	0
Elymus giganteus	2	2	1	274	0	0
Elymus glaucus	2	2	2	167	0	0
Elymus junceus	1	1	1	101	0	0
Elymus triticoides	3	5	2	320	0	0
Festuca idahoensis	1	1	1	94	0	0
Hordeum brevisubulatum	1	2	1	289	0	0
Hordeum bulbosum	2	3	1	698	0	0
Hordeum jubatum	1	1	1	281	0	0
Hordeum nodosum	2	3	1	402	0	0
Secale montanum	2	2	1	813	0	0

Attempts to hybridize cultivated oats with grass species have been very limited except with *Arrhenatherum elatius*. The oat varieties used were Markton, Red Rustproof, and Winter Turf; all were used with *A. elatius*. A summary of the results is given in table 5. No seeds appearing to vary from the normal were observed, and no crosses were successful.

TABLE 5.—*Summary of results of artificial pollination in attempted hybridization of common oats (Avena sativa and A. byzantina) with other grass species*

Grass species	Period of study	Oat varieties	Grass strains	Total florets	Hybrid seeds	Hybrid plants
	Years	Number	Number	Number	Number	Number
Arrhenatherum elatius	2	3	1	411	0	0
Bromus inermis	1	1	1	43	0	0
Dactylis glomerata	1	1	1	96	0	0
Elymus canadensis	1	1	1	68	0	0
Koeleria cristata	1	1	1	28	0	0

DISCUSSION

Various workers, including Armstrong (*4*), Veruschkine and Shechurdine (*40*), and Kikot and Volkova, according to Tzitzin (*35*), have indicated that success in crossing depends primarily on the parental strains used. Armstrong found two *Agropyron elongatum* types to be quite different in compatibility with wheat. Kikot and Volkova stated further that individual plants within a strain might vary greatly in this respect. The present study has indicated substantiation of this for the female parents. Similar differences in relative compatibility of male parents might be expected to exist. Negative results from attempted crosses should be regarded, therefore, as applying only to the strains used, and not as establishing the complete incompatibility of the species.

In consideration of results of investigations, possible differences in botanical nomenclature should be recognized. While for certain species agreement in identity may be assumed, this would not apply in all instances. The relation of *Agropyron intermedium*, *A. obtusiusculum*, and *A. glaucum* may be synonymous. However, the *A. glaucum* strain used by Armstrong (*4*) and Johnson (*10*) is not thought to be closely similar to the *A. intermedium* used by the present writer. Specific distinction in such polymorphic forms as *A. repens* is also subject to much individual interpretation.

The results of crossing are in general agreement with those previously reported. All of the wheat-grass crosses obtained have been made by other workers. The possible compatibility of certain races of *Agropyron repens* with *Triticum aestivum* was indicated by Tzitzin (*32*), though again attempts to make this cross have been unsuccessful. In the present studies *T. aestivum* and *S. montanum* were not compatible. The influence of parental strains utilized suggests itself as a possible factor in the results obtained.

So far as the writer knows the crosses *Secale cereale* × *Agropyron repens*, *S. cereale* × *A. sibiricum*, and *S. cereale* × *A. trichophorum* have not been reported in earlier literature. The hybrid *S. cereale* × *A. intermedium* obtained by Veruschkine and Shechurdine might be very

similar to the last-named cross, however, since *A. trichophorum* is considered by these workers to be closely related to *A. intermedium*. The hybrid of Ljubimova *(18)* might be considered in this group. Tzitzin *(35–a)* also referred to "rye-couch grass hybrids." Intergrading biotypes between *A. intermedium* and *A. repens* may also exist. The fact that the three species previously mentioned have been reported to have the same chromosome number conforms with observed morphological similarities as a basis for their possibly close relationship.

While no grass species were crossed successfully with *Hordeum vulgare*, the partial development of the barley caryopsis following pollination with certain ones suggests that some incomplete compatibility may exist. This development was particularly noticeable in the results of pollinating *H. vulgare* × *H. bulbosum* but was also observed in other hybrid combinations. The recent results of Quincke *(26)* suggest the problem of the relative merits of reciprocal crosses in obtaining hybrids successfully.

SUMMARY

Hybridization of wheat (*Triticum aestivum* L.) was attempted with other grasses, including 15 species of *Agropyron*, 7 of *Elymus*, 2 of *Festuca, Lolium perenne*, and *Secale montanum* as pollen parents.

Certain hybrid plants were obtained only in the following crosses: *Triticum aestivum* × *Agropyron elongatum; T. aestivum* × *A. intermedium;* and *T. aestivum* × *A. trichophorum.*

Rye varieties (*Secale cereale* L.) were studied in combinations with nine species of *Agropyron*, seven of *Elymus*, and *Bromus inermis, Hordeum bulbosum*, and *Secale montanum.* The following crosses were successful: *Secale cereale* × *Agropyron repens, S. cereale* × *A. sibiricum, S. cereale* × *A. trichophorum*, and *S. cereale* × *S. montanum.*

Five *Agropyron*, two *Bromus*, nine *Elymus*, and four *Hordeum* species, and *Festuca idahoensis* and *Secale montanum* were used as male parents in crosses with *Hordeum vulgare* L. No hybrids were obtained.

Limited attempts to hybridize cultivated oats (*Avena sativa* L. and *A. byzantina* C. Koch) with other grasses, particularly *Arrhenatherum elatius*, were unsuccessful.

Hybrid plants studied have proved to be sterile in all crosses except those derived from *Secale cereale* × *S. montanum.*

All varieties used in wheat × grass and rye × grass crosses were not equally successful, results varying with the strains utilized as female parents. Inability to produce particular strain combinations between species should not be considered as proof of complete incompatibility of the species in intergeneric crosses.

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SOME ASPECTS OF THE PHYLLOTAXY OF TOBACCO [1]

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INTRODUCTION

The genus *Nicotiana*, which includes the true tobaccos, is characterized by an alternate disposition of the leaves throughout. An abnormal condition may sometimes bring about an occasional pair of opposite or whorled leaves in some varieties of tobacco, more especially in the rather anomalous short-day variety known as Maryland Mammoth, but this is too exceptional to be considered in any study of the phyllotaxy of tobacco.

HOW TO MAKE THE COUNT TO DETERMINE THE LEAF ARRANGEMENT

In order to determine the number of leaves present, counting from a certain leaf or node to the next leaf directly in line above the first one, the leaves counted must include the next younger leaf in proper order and all intervening leaves, but not the first leaf from which the count started. In this count it is very important to determine the true direction of the spiral, which may run to the left or to the right. This is found by passing to the nearest leaf above, which will be the next younger leaf from the starting point. The course is thus continued up the stem until the leaf directly above the first one is reached. In making this count, the number of encirclements of the stem is noted also. The phyllotaxy found is expressed as a fraction in which the numerator represents the number of encirclements of the stem and the denominator the number of leaves counted, as 2/5, 3/8, or 5/13.

CONSTANCY OF A PARTICULAR ARRANGEMENT

To determine the more fixed leaf arrangement in tobacco, it is well to study the leaf relations as far down the stalk as practicable. This is true for the reason that the morphological plan of the stem and leaf arrangement appears to be determined in the transition zone from root to shoot in the cotyledonary region of the embryonic plant. A count here, then, would more correctly express the inherent or primitive morphology of the plant than would a study of the secondary suckers or the top of the plant, which is farthest removed from the basal transition zone and more closely related to external forces governing flowering, fruiting, and senescence of the original plant.

While some tobacco plants show a constant leaf arrangement from the base to the top, others may change to a different fractional expression, usually of a lower order but sometimes of a higher order. As a matter of fact, tobacco shows a more or less inconstant phyllotaxy comparable to that of *Actinomeris alternifolia* (L.) DC., *Verbesina occidentalis* (L.) Walt., and many species of sunflower, including

Helianthus tuberosus L. This behavior is not anomalous, but in reality is usual for many plants, and it would appear to be the normal expression for some strains of tobacco as well. These changes of leaf arrangement have been found in a number of commercial types.

While it may be true that many features of the plant are predetermined in the original embryonic axis, it would be absurd to conclude that every branch, sucker, or all the new shoots arising when the plant is cut to the ground are an exact unfolding of some preformed embryonic prototype simply expanded to natural size. Life is very plastic, and its primitive plans in many instances may be greatly modified by conditions of habitat.

DIRECTION OF THE SPIRAL

If one will take any tall stem, such as that of tobacco, having many successive nodes, or points of leaf insertion, fasten the end of a string to one node, as shown in figure 1, and follow up the stem in the proper course to the successively younger leaves from node to node, he will at once fix the left or right course of the spiral.

If the inclines of the string are to the right, the winding, as seen down the length of the stem, is counterclockwise; if the inclines are to the left, the winding is clockwise. These relations are shown in figure 1.

The direction of this spiral is one of the most fixed characters of the plant. While the actual leaf arrangement may change with respect to the number of leaves on the stem between points in line, the spiral course, probably originating somewhere in the transition zone between root and stem, is invariably maintained throughout in the normal plant and holds its primitive direction regardless of environmental influences. The writer has found no tobacco plant in any variety where this spiral course has reversed itself at any point, even in the suckers following topping or cutting back. Every variety shows both courses in different proportions and where selection for this character has not been followed the percentage of clockwise and counterclockwise nodes approaches a 50-percent ratio, which is one of normal chance where these two alternatives alone obtain. There is no reason, however, why strains could not be selected where ratios of one spiral pattern to the other would be very high, or even where either pattern, perhaps, might occur with a 100-percent certainty. When considered with other characteristics, this might serve, in some instances, to identify a certain strain.

Figure 1.— Different leaf arrangements found in some varieties of tobacco in the lower and upper parts of the main stem. The white string passes from the lowest leaf to the next younger leaf above, being fastened to each leaf node by a black tack, except to the nodes in a direct line above the first leaf, where large-headed roofing nails are used, the heads of which are painted white. *A–C*, Robinson Maryland Medium Broadleaf. *A*, 5/13 below, changing to 3/8 and 2/5 above; spiral course up the stem, counterclockwise. *B*, 5/13 below, changing to 3/8 above; spiral course, clockwise. *C*, 5/13 below, changing to 2/5 above; spiral course of leaves, counterclockwise. *D*, Rawling Medium Maryland Broadleaf, 5/13 below, 3/8 above; spiral course, clockwise. *E*, Maryland Mammoth, 5/13 below, changing to 3/8 above; spiral course, clockwise. *F*, Standup Maryland Mammoth, 5/13 throughout; spiral course, clockwise. *G*, Wilson Maryland Broadleaf, 3/8 below, changing to 2/5 above; spiral course, counterclockwise. *H*, Maryland Connecticut Broadleaf, 3/8 throughout; spiral course, clockwise. *I*, One Sucker, 2/5 throughout; spiral course, counterclockwise.

LEAF ARRANGEMENT OF SOME COMMERCIAL VARIETIES AND SPECIES

Table 1 shows the leaf arrangement and spiraling course of 43 Maryland Mammoth plants grown in the field at the Arlington Experiment Farm, Arlington, Va., in 1940. It will be seen here that tobacco plants not infrequently change their phyllotaxy in different portions of the stem.

TABLE 1.—*Phyllotaxy of 43 plants of Maryland Mammoth tobacco as noted from bottom to top of stem*

Phyllotaxy	Plants		Phyllotaxy	Plants	
Arrangement of leaves·	*Number*	*Percent*	Direction of spiral	*Number*	*Percent*
5/13 throughout_____	12	27. 6	Clockwise _____	20	46. 5
3/8 throughout___	8	18 6	Counterclockwise __ __	23	53 5
5/13 below, 3/8 above____	16	37 2			
3/8 below; 5/13 above_ ___	4	9. 3			
5/13 below and above; 3/8 middle _ _____	2	4. 6			
3/8 below and above; 5/13 middle_____ _____	1	2. 3			

Assuming that the arrangement of leaves on the lower part of the stem expresses the more primitive condition, this group of plants showed 69.4 percent with a 5/13 arrangement and 30.2 percent with a 3/8 arrangement.

In one plant of this group a few leaves were practically opposite or at the same node, and there seemed to be an accidental or anomalous phyllotaxy of 2/7 or 3/11. The clockwise and counterclockwise spiraling of the leaves, namely, 46.5 percent and 53.5 percent respectively, in this group of plants represented a nearly equal numerical occurrence.

In table 2 there is presented the phyllotaxy, or leaf arrangement and spiral course around the stem, of a number of commercial Ameri-

TABLE 2.—*Phyllotaxy of the leaves on the main stem below and suckers of some commercial American tobaccos and other varieties and species*

Variety or species	Total plants	Plants having indicated leaf arrangement						Spiral course				Phyllotaxy of suckers and related data
		2/5		3/8		5/13		Clock-wise		Counter-clock-wise		
	No	*No.*	*Pct*	*No.*	*Pct.*	*No*	*Pct*	*No*	*Pct.*	*No*	*Pct*	
Ambalema (South American).	6	0	0	6	100	0	0	1	16 6	5	83 3	A commercial variety in Argentina No suckers present as a result of topping
Catterton Maryland Broadleaf.	24	0	0	24	100	0	0	11	45. 8	13	54 1	May have been a selection from Johnson Root Rot Resistant × Maryland Connecticut Broadleaf No suckers studied
Little Dutch ___ ___	8	0	0	8	100	0	0	5	62. 5	3	37. 5	All below showed a 3/8 phyllotaxy; 1 plant appeared to change to 2/5 above
Little Orinoco_ ____	3	0	0	3	100	0	0	3	100	0	0	1 plant 3/8 below, but a tall sucker from topped portion above seemingly 2/5 below, 1/3 above, with spiral course clockwise throughout ¹ plant 3/8 to middle, 2/5 to top, and sucker from topped portion 3/8, and counterclockwise

TABLE 2.—*Phyllotaxy of the leaves on the main stem below and suckers of some commercial American tobaccos and other varieties and species*—Continued

Variety or species	Total plants	Plants having indicated leaf arrangement						Spiral course				Phyllotaxy of suckers and related data
		2/5		3/8		5/13		Clockwise		Counter-clockwise		
	No.	No.	Pct	No.	Pct	No.	Pct	No.	Pct	No.	Pct	
Maryland Connecticut Broadleaf	35	0	0	35	100	0	0	21	60	14	40	2 plants with top suckers 3/8 on all, and same spiral arrangement as on main stem.
Maryland Mammoth	23	0	0	16	69.6	7	30 4	---	---		..	1 plant 3/8 below, 5/13 above on the main stem
Maryland Standup Resistant	20	0	0	20	100	0	0	9	45	11	55	Johnson Root Rot Resistant Burley × Maryland Connecticut Broadleaf. No data on suckers, as these had been removed.
Nicotiana glauca	5	0	0	5	100	0	0	4	80	1	20	Young plants not yet in flower studied
Nicotiana gossei (Australian)	24	20	83.3	4	16.7	0	0	¹6	33 3	12	66 6	Some untopped plants with 2 or 3 suckers, but the suckers showed same phyllotaxy as the main stem and the same spiraling throughout
Nicotiana rustica Olson strain	11	0	0	11	100	0	0	6	54 5	5	45.4	Plants mature in the field
One Sucker	4	2	50	2	50	0	0	3	75	1	25	1 plant with 3/8 phyllotaxy, changed to 2/5 above; spiral arrangement clockwise throughout.
Rawling Fusarium Resistant	34	0	0	29	85 3	5	14 7	18	52 9	16	47	1 plant with divided main stem below, both 3/8, with counterclockwise arrangement as in case of main stem on both branches (Strain of Maryland Medium Broadleaf.
Robinson Maryland Medium Broadleaf	44	4	9 1	33	75	7	15 9	21	60	14	40	1 plant with bifurcated stem 3/8 phyllotaxy and clockwise arrangement on both branches
Robinson Maryland Medium Broadleaf (pure line)	32	0	0	13	40 6	19	59.4	_	_	_	_	1 plant with 3/8 phyllotaxy on main stem produced 2 suckers, each with 3/8 phyllotaxy. 1 plant with 5/13 phyllotaxy on main stem produced 2 suckers above, each with 3/8 phyllotaxy
Samsun (Turkish)	4	0	0	4	100	0	0	4	100	0	0	All 4 plants with 3/8 phyllotaxy below and each with 1 sucker 2/5 above and clockwise throughout
Standup Maryland Mammoth	46	0	0	28	60 9	18	39 1	_	_	_	_	White Burley × Maryland Mammoth No suckers present
Wilson Maryland Broadleaf	41	0	0	37	90 2	4	9 8	17	41 4	24	58 5	1 plant bifurcated below showed 3/8 phyllotaxy and counterclockwise arrangement on both branches
Xanthi (Turkish)	4	4	100	0	0	0	0	2	50	2	50	1 plant 2/5 below on main stem, changing to 3/8 at top, clockwise throughout. 1 plant 2/5 below, 3/8 above, with a top sucker 1/3 phyllotaxy, clockwise on main stem and suckers. 1 plant 2/5 below, apparently 3/8 above, with 1 sucker above 2/5 and counterclockwise throughout

¹ 18 plants observed as to spiral course

can tobacco varieties, together with other varieties grown elsewhere commercially, and also data for the species *Nicotiana gossei* Domin., *N. glauca* Graham, and *N. rustica* L. The last-named is no longer grown in America for its leaf but is being tested for nicotine production because of its very high content of that alkaloid.

The data in table 2 are sufficient to indicate that the leaf arrangement on the lower part of the main stem of most of our commerical American tobaccos is expressed by the fractions 3/8 and 5/13. A 2/5 phyllotaxy is not usual, but has been found in a few plants of Robinson Maryland Medium Broadleaf; in One Sucker; in Xanthi, a Turkish variety; and in *Nicotiana gossei*, a little-known wild Australian species. The last-named species was characterized by a high 2/5 dominance, namely, 83.3 percent in a lot of 24 plants. There was no 5/13 occurrence in this species but 16.7 percent of the plants showed a 3/8 phyllotaxy.

Robinson Maryland Medium Broadleaf, represented by a progeny of 32 plants obtained from seed of 1 plant, gave the highest percentage of the 5/13 phyllotaxy, namely, 59.4 percent.

Both *Nicotiana glauca* and *N. rustica* had a leaf arrangement expressed by 3/8.

In most of these species and varieties of *Nicotiana*, the course of the spiraling on the stem was noted, and usually this was very variable. In one the counterclockwise arrangement was most frequent; in another the clockwise arrangement. Since there has been no conscious breeding or selection for this character, a condition of variability would naturally be expected.

In table 2, attention has been directed chiefly to the leaf arrangement on the lower part of the main stem, with the inference that the more primitive phyllotaxy would be more correctly represented there. Many tobacco plants change their phyllotaxy, however, and it would seem that the change is usually to a lower order of the primary series rather than to a higher order. Suckers may or may not continue the same fractional arrangement found on the main stem. A 1/3 fractional arrangement has been found on some suckers, but this has never been observed on the main stem of any tobacco, although conceivably it may occur.

SUMMARY AND CONCLUSIONS

A general survey of phyllotaxy in tobacco is presented.

There are several normal leaf arrangements in varieties and species of *Nicotiana*, those on the main stem being expressed by the fractions 2/5, 3/8, and 5/13, where the numerator indicates the number of encirclements around the stem and the denominator the number of leaves encountered beginning with a given leaf and passing upward to successively younger leaves around the stem until a leaf directly in line with the first is reached. The count begins with the next leaf above the starting point and continues to and includes the next leaf alined with the one at the starting point.

Tobacco may present a 1/3, 2/5, 3/8, or 5/13 arrangement of the leaves. The 1/3 arrangement has been found only in the suckers of a very few plants of those normally showing the low fraction order 2/5, although severe cutting back or maltreatment that produces abnormal growth and branching may be expected to produce 1/3,

2/5, or 3/8 arrangements in any plant where the main stem shows a more complex phyllotaxy.

The 2/5 phyllotaxy is not usual in the large-leaved American varieties but appears to be the prevalent form in some small-leaved Turkish varieties, such as Xanthi.

A 3/8 and a 5/13 phyllotaxy are the common arrangements of the leaves in tobacco, these occurring in varying proportions. There is no reason, however, why breeding and rigid selection should not establish a high frequency of any phyllotaxy normal to the tobacco plant. It is possible that the phyllotaxy expressed by the fraction 5/13 represents a higher or more recent development in tobacco, more especially since a lower or simpler order may be enforced by changes in the age of the stem or branches, or by mutilations such as cutting down or back, by this means enforcing rejuvenation of growth and expressions farther removed from the original and more primitive phyllotaxy nearest the earlier embryonic tissues of the main stem.

As in many other plants, the phyllotaxy of tobacco is more or less variable, owing to internal or external factors. The more constant or primitive arrangement is most likely to occur on the lower part of the main stem rather than near the inflorescence or on suckers following cutting or topping.

The spiral arrangement upward to successively younger leaves may express itself as a clockwise or a counterclockwise course. This is a much more rigidly enforced relationship in the constitution of the plant than the fractional phyllotaxy encountered. While the fractional expressions may change on different parts of the stem or in the branches or suckers, the spiral course once established remains fixed to a very high degree. It probably never reverses itself except with very abnormal torsions, and this has not been observed by the writer.

RELATION OF ASCORBIC ACID CONCENTRATION IN JUICE OF FLORIDA GRAPEFRUIT TO VARIETY, ROOTSTOCK, AND POSITION OF FRUIT ON THE TREE [1]

By Paul L. Harding, *horticulturist*, and Edward E. Thomas, formerly *minor scientific helper, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture* [2]

INTRODUCTION

During recent years much interest has been shown in the nutritive values of different foods in both human and animal diets. In part, at least, this increasing interest has resulted from the chemical identification of some of the common vitamins and the development of accurate and rapid methods for determining the vitamin content of various foods and food products.

The seasonal changes in the ascorbic acid content of the juice of Florida oranges have been reported by Harding, Winston, and Fisher.[3][4] These authors pointed out that no correlation was found between the ascorbic acid content of orange juice and its quality as judged by taste. They also reported a significant difference in ascorbic acid concentration between fruit exposed to the sun and shaded fruit, the exposed fruit having the higher ascorbic acid concentration.

The investigation reported herein had the following objectives: (1) To determine the ascorbic acid content of the juice of Florida grapefruit (*Citrus grandis* (L.) Osbeck), and (2) to determine whether the ascorbic acid content is influenced by rootstocks, variety, and the position of the fruit on the tree (outside or exposed fruit compared with inside or shaded fruit).

MATERIAL AND METHODS

The grapefruits used in this study were collected at random from the "regular bloom" of mature trees located in a commercial grove on Merritt Island, Fla. (east coast). The trees had slightly sparse but normal foliation typical of the section. The fruits were from three different plots of five trees each: Marsh grapefruit grown on rough lemon rootstock, Marsh grapefruit grown on sour orange rootstock, and Foster (pink) grapefruit grown on sour orange rootstock.

Twenty-six fruits were picked from each of 15 trees: 13 fruits from outside branches well exposed to sunlight, and 13 from inside partially shaded branches. They were picked on November 10, 1939, and placed in storage at 32° F. at the United States Department of Agriculture Horticultural Field Laboratory, Orlando, Fla., and were analyzed within a few days.

The ascorbic acid or vitamin C content of the juice was determined individually for each of the 13 outside and of the 13 inside fruits from each of the 5 trees in the 3 plots, or for 390 fruits. In making the determinations, the juice was extracted by hand squeezing and then strained to remove pulp and seeds. Determinations were made by the method described by Bessey and King,[5] which consists essentially of the titration of grapefruit juice with a solution of freshly prepared sodium 2,6-dichlorobenzenoneindophenol (2,6-dichlorophenolindophenol) which had been standardized against fresh commercial crystalline ascorbic acid.

RESULTS

In table 1 are given the ascorbic acid concentration (milligrams per milliliter of juice) in 390 individual fruits, the total in the samples from the 13 fruits from each exposure on each tree, and the average amount in a milliliter of juice. Totals and means calculated from the individual fruit determinations in table 1 are presented in table 2. The data are analyzed statistically [6] [7] [8] in tables 3 and 4.

The determinations indicate that Florida grapefruit is a rich source of ascorbic acid. The values were in close agreement with unpublished data from other producing districts in the State. On a milligram per milliliter of juice basis, the values reported for grapefruit in table 1 are slightly lower than those that have been reported for Florida oranges [9] for comparable dates. However, since grapefruit is generally much larger than oranges and is usually served in halves, it is quite possible that the total ascorbic acid consumed by one person at a meal would be similar to that in an ordinary serving of orange juice.

[5] BESSEY, OTTO A., and KING, C. G. THE DISTRIBUTION OF VITAMIN C IN PLANT AND ANIMAL TISSUES AND ITS DETERMINATION. Jour. Biol. Chem. 103 687–698. 1933.
[6] BARTLETT, M S., and GREENHILL, A W. THE RELATIVE IMPORTANCE OF PLOT VARIATION AND OF FIELD AND LABORATORY SAMPLING ERRORS IN SMALL PLOT PASTURE PRODUCTIVITY EXPERIMENTS Jour. Agr. Sci. [England] 26: [258]–262 1936.
[7] FISHER. R. A. THE DESIGN OF EXPERIMENTS 252 pp Edinburgh and London. 1935.
[8] SNEDECOR, GEORGE W. STATISTICAL METHODS APPLIED TO EXPERIMENTS IN AGRICULTURE AND BIOLOGY. Ed 3, 422 pp. Ames, Iowa. 1940.
[9] See footnote 3, p. 57.

TABLE 1.—*Ascorbic acid content of the juice of fruits produced on outside and inside branches of Marsh and Foster grapefruit at Merritt Island, Fla., Nov. 10, 1939*

Variety and rootstock	Ascorbic acid per milliliter of juice of individual fruits from—									
	Outside branches of tree No. —					Inside branches of tree No. —				
	1	2	3	4	5	1	2	3	4	5
	Mg.	Mg	Mg.	Mg	Mg.	Mg.	Mg.	Mg	Mg.	Mg.
Marsh on rough lemon rootstock	0.41	0 40	0 53	0 48	0 47	0 41	0 41	0.48	0 49	0 48
	40	.40	.50	.47	.46	40	.39	.47	.48	.47
	.40	.40	.50	.47	46	.38	.39	.47	47	.46
	.40	39	.49	47	.44	.37	37	.46	47	.45
	39	38	47	.46	44	36	37	.45	.45	.45
	39	.38	47	.45	44	.36	37	.45	.45	.44
	39	38	.46	45	.44	35	.36	.44	44	.44
	.39	37	.46	45	.44	35	.36	.41	.44	.43
	37	37	.46	.45	.43	35	.36	.44	.42	.43
	.37	.37	16	44	.43	.35	34	.44	.42	.43
	37	36	.46	.44	.43	34	.33	.44	42	.43
	34	35	.45	.44	.43	34	.33	42	.40	.41
	.33	35	.44	.43	.41	33	.32	.41	40	.40
Total	4 95	4 90	6 15	5 90	5.72	4 69	4.70	5 81	5 75	5 72
Average	.38	38	47	.45	.44	.36	.36	45	44	.44
Marsh on sour orange rootstock	47	44	41	.42	43	.43	.39	41	41	.40
	44	44	41	.41	.42	.41	.38	.39	38	.40
	44	43	.39	41	.42	40	37	38	37	39
	.44	41	.39	.41	41	40	37	.38	.37	.38
	44	41	.38	40	41	39	37	38	37	38
	44	41	38	40	41	39	36	37	.36	.38
	43	40	38	40	40	39	36	.36	36	.37
	42	.39	38	.40	.40	37	34	.36	35	37
	42	39	38	39	40	37	33	36	35	.37
	42	39	.37	.39	.39	37	.33	35	35	.36
	.41	.39	.37	39	.37	37	32	.35	.35	.36
	39	.38	37	.38	.36	.36	32	34	34	.35
	39	37	36	.38	.35	35	.32	34	.34	35
Total	5 55	5 25	4 97	5.18	5 17	5 00	4 56	4 77	4 70	4.86
Average	43	40	38	.40	.40	.38	.35	37	36	37
Foster (pink) on sour orange rootstock	46	.46	.44	.46	.48	42	.45	47	.46	.43
	44	.46	.44	.46	47	.41	.43	44	44	.43
	.44	.44	43	.46	.46	41	.42	.43	44	.42
	44	.44	.43	.45	44	.41	.42	.41	44	.42
	.44	.43	.42	.44	44	.39	.41	.41	.42	.41
	43	43	42	.43	43	39	.41	.41	.42	.41
	.41	43	.42	.42	.43	38	.40	.40	41	.41
	40	.42	41	41	.41	.38	.40	.39	41	.40
	40	39	41	.41	41	.38	.40	39	.40	.40
	39	.39	.40	41	.41	37	.39	.39	39	.40
	.39	38	40	41	.39	.37	.36	.38	.39	.40
	.39	.37	.40	40	37	.36	36	.38	.37	.39
	.38	.34	39	.39	36	.33	.34	.38	.36	.37
Total	5 41	5 38	5 41	5 55	5.50	5 00	5 19	5 28	5 35	5.29
Average	.42	.41	.42	43	.42	38	.40	.41	.41	.41

TABLE 2.—*Total and mean ascorbic acid determinations calculated from individual fruit determinations listed in table 1*

Variety and rootstock	Total (per 65 ml. of juice) from fruit from indicated position		Total (per 130 ml. of juice)	Mean (per 13 ml. of juice)
	Outside	Inside		
	Milligram	*Milligram*	*Milligram*	*Milligram*
Marsh on rough lemon rootstock	27 62	26. 67	54 29	5 43
Marsh on sour orange rootstock	26 12	23. 89	50 01	5 00
Foster on sour orange rootstock	27 25	26 11	53 36	5 34
Total (per 195 ml. of juice)	80 99	76 67		
Total (per 390 ml of juice)			157. 66	
Mean (per 13 ml. of juice)	5. 40	5 11		5. 26

TABLE 3.—*Analysis of variance of the ascorbic acid determinations listed in table 1*

Variance due to—	Degrees of freedom	Sum of squares	Mean square	Variance ratio [1]
Sources	2	0 0780	0 0390	2 10
Within sources (between trees within sources)	12	2236	0186	
Total whole trees	14	3016	0215	
Positions	1	0479	0479	59 88**
Position×source	2	0073	.0037	4 62*
Position×tree within sources	12	0100	0008	
Total half trees	29	.3668	0126	
Within half trees	360	2010	0006	
Grand total	389	.5678		

[1] ** = Highly significant. $P=0.01$ (odds 99 1). * = Significant, $P=0.05$ (odds 19 1).

TABLE 4.—*Analysis of the variance of the ascorbic acid concentration of fruits of Marsh grapefruit grown on rough lemon and sour orange rootstocks and of those of Foster and Marsh grapefruit grown on sour orange rootstock*

MARSH GRAPEFRUIT ON ROUGH LEMON AND SOUR ORANGE ROOTSTOCK

Variance due to —	Degrees of freedom	Sum of squares	Mean square	Variance ratio [1]
Between sources	1	0 9159	0 9159	2 59
Trees within sources	8	2 8340	3543	
Positions	1	5056	5056	37 45**
Position×source	1	0820	0820	
Position×trees within sources	8	1078	0135	
Total	19	4 4453		

FOSTER AND MARSH GRAPEFRUIT ON SOUR ORANGE ROOTSTOCK

Between sources	1	0 5612	0 5612	15 85**
Trees within sources	8	2834	0354	
Positions	1	5679	5679	46 55**
Position×source	1	0593	0593	
Position×trees within sources	8	0979	.0122	
Total	19	1 5697		

[1] * * = Highly significant; $P=0.01$ (odds 99 1).

The analysis of variance of the ascorbic acid determinations (tables 3 and 4) shows a highly significant difference between the two position means (determinations on juice from fruit produced on outside and inside branches) and (table 4) between Foster and Marsh grapefruits on sour orange rootstock (determinations on juice from fruits produced on different varieties). The ascorbic acid concentration in fruit produced on the inside branches was lower than that of fruit produced on the outside branches.

There was a significant interaction between position and source. The differences between positions were not proportional in the three sources. This, however, is of minor importance in view of the highly significant difference due to positions. The results are of particular interest because the trees were sparsely foliated and the inside fruit was but partly shaded.

In table 4 the analysis of variance was made to determine the difference in ascorbic acid concentration in Marsh grapefruit grown on rough lemon and sour orange rootstocks. The ascorbic acid concentration was slightly higher in the fruit grown on rough lemon. These differences, however, were not significant.

Table 4 presents also an analysis of variance of determinations made on the juice of Foster and Marsh grapefruit both grown on sour orange rootstock. These data indicate a highly significant difference ($P=0.01$) between the two varieties; the Foster variety had the higher ascorbic acid concentration.

SUMMARY

The ascorbic acid concentration was determined for 390 individual grapefruits picked from outside and inside branches of 5 trees each of Marsh grapefruit grown on rough lemon rootstock, of Marsh grapefruit grown on sour orange rootstock, and of Foster (pink) grapefruit grown on sour orange rootstock.

The results showed that Florida grapefruit from various sources has high antiscorbutic properties. In this experiment the average ascorbic acid concentration was 0.40 milligram per milliliter of grapefruit juice.

A relatively small but consistent difference was found in the ascorbic acid concentration in fruits obtained from outside and inside branches from the three sources; the outside fruit had the higher ascorbic acid concentration. This difference was found to be significant at the 1-percent level.

The ascorbic acid concentration in fruits from the Marsh variety grown on rough lemon and on sour orange rootstocks was determined. A statistical study of these data showed a higher ascorbic acid content per milliliter of juice of fruit grown on rough lemon rootstock, but the difference was not significant.

The ascorbic acid content of juice of fruits on the Marsh and Foster (pink) varieties of grapefruit, both of which were grown on sour orange rootstock, was also determined. The analysis of variance of these data indicates a higher ascorbic acid concentration in the Foster fruit. This difference was found to be highly significant ($P=0.01$).

HYBRID VIGOR AND WEIGHT OF GERMS IN THE SEEDS OF MAIZE [1]

By J. H. KEMPTON, *botanist*, and JOHN W. McLANE, formerly *assistant biophysicist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

The stimulation of growth resulting from hybridization remains one of the unexplained biological facts. The fact itself has been recognized from very early times and is the basis of the current effective system for improvement in maize and other crop plants. But just what takes place in the union of unlike germ plasms that brings about the observed stimulation has not been determined. The exact nature of the reaction between diverse hereditary elements is of great theoretical as well as practical interest, and until this problem has been solved all facets of hybrid vigor will continue to merit attention.

PREVIOUS INVESTIGATIONS

The demonstration of hybrid vigor in resting embryos, as distinct from seed size, was made by Ashby (1, 2).[2] Using maize as his test plant, he investigated the possibility that the increased size of the mature hybrid organism over that of its parents was determined by the weight of the embryo and not by an increased rate of growth of the developing hybrid plant over that of the parents. Ashby concluded that hybrid corn plants grew at the same rate as their more rapidly growing parent and that their larger mature size followed from the fact that they had a larger embryo to start with, i. e., more capital invested. He subsequently extended his studies to other species and reached the same conclusions (3).

Ashby's conclusions as to the nature of hybrid vigor have been criticized by East (7) and amplified by Ashby (4, 5). There can be no difference of opinion as to the facts. Hybrid embryos are often heavier than those of their parents. The divergence of opinion comes with the question as to whether all the increase in mature size of a hybrid over its parents can be attributed to the initial advantage of a larger embryo and, therefore, to a higher rate of growth, limited to the period before dormancy.

Obviously, if hybrid embryos produced on inbred plants are heavier than inbred embryos on the same or sister plants, they must grow either for a longer period or at a more rapid rate if growth is under-

[1] Received for publication May 27, 1941.
[2] Italic numbers in parentheses refer to Literature Cited, p. 80.

stood to mean an increase in dry weight, whether resulting from larger cells or more of them.

Lindstrom (*8*), using mutilated maize plants and failing to grasp Ashby's main thesis, believed he had demonstrated that the larger size of hybrid plants was not the result of a greater initial capital.

Sprague (*12*) showed, with some of the same seed stocks as Ashby, that reciprocal crosses differing in germ size attained identical final weights. He also pointed out the fallacy of Lindstrom's reasoning and reported a cross where the embryos were no heavier than those of one of the parent inbreds, yet the final weight of the hybrid plant was twice that of the inbred plant. Sprague concluded that the rate of growth of hybrids in the earlier stages of development was more rapid than that of the inbreds and that the increased final size was compounded of a more rapid growth rate and a larger initial embryo size. He believed his experiments were more readily explained on the basis of dominant favorable genes than on the more subtle physiologic stimulation resulting from the union of unlike germ plasms.

Paddick and Sprague (*10*), experimenting with maize, measured the relation of increase in hybrid germ weight, over that of the female parent, to the resulting forage and grain weights. Twenty-four hybrids were tested in 1 year and 22 in another. There was a low correlation of germ-weight increase with grain yield both years ($r=0.42$ and $r=0.36$) but no correlation with yield of stover.

These authors also measured the variation of kernel parts of an inbred line of maize and of several hybrids with it. They pointed out that, irrespective of seed size (which varies with position on the ear and with years), the ratio of germ to endosperm remains relatively constant.

Evidence was also presented of very great differences between reciprocal crosses in weight of seed parts. Of the nine reciprocal crosses tested, there were five hybrids that had germs lighter in weight than those of the female parent, and four of these were below both parents in germ weight. These hybrids, therefore, although presumably giving the customary mature-plant increases in weight, showed no evidence of heterosis in their germs.

Passmore (*11*), working with *Cucurbita pepo*, where seed size and embryo size correspond and there are no complications with endosperm, showed that where reciprocal F_1 hybrids differed by two to three times in germ weight the resulting plants were not greatly different at maturity. However, the heavier seeds gave a decided initial advantage, which became less with time. The final differences were equalized partially, because the plants from the small embryos matured later. Passmore made no direct statement as to the effect of heterosis on embryo size but left the inference that there was none. She stated, "Since the size of the F_1 seed is determined by the maternal parent, it is possible, in a reciprocal cross, to get two hybrids which are genetically identical but which have widely different seed sizes." Probably, in view of the context, the determination of seed size is not wholly by the maternal parent, as it would seem strange were the effect of hybrid vigor not exhibited in the embryo of squashes.

Murdoch (*9*), improving on Ashby's technique of dissection of the maize embryo, confirmed the results of Ashby as to weight of embryos but apparently overlooked Sprague's results, which first proved that

hybrids did not always have heavier embryos than their parents. He believed that one reason for the lack of accord between Ashby and his critics resulted from a failure to understand what is meant by the term "embryo." For his study, which was limited to embryo size, Murdoch removed the scutellum, the coleorhiza, and the endosperm.

It is true that the scutellum, coleorhiza, and endosperm do not become part of the mature corn plant, and for the purpose of calculating the rate of growth of the plant following germination these parts should not be considered as capital tissue. However, from the standpoint of the effect of hybrid vigor, each of these parts should respond alike to heterosis, as they are the products of fertilization. There is a cytological reason for discounting the response of the endosperm to cross-pollination, because the female parent plays a disproportionate part in the development of this tissue, furnishing two sets of genes to one from the male. In the case of the embryo tissue, comprising the scutellum, coleorhiza, and the embryo proper, both parents contribute equally, and indeed the scutellum and the coleorhiza are as much parts of the embryo as are the leaf and root primordia. They do not increase in cell number as the seedling expands and in a strict sense are not part of the capital. Their size, however, at the resting stage must be considered as much the result of heterosis as that of the primordia which are to become the next stages of the plant. It should be kept in mind that the cells of the mesocotyl (epicotyl), coleoptile, and indeed those of part of the leaves and roots, are formed before the resting period, and that the elongation of these organs, together with some part of their increase in dry matter in the early stages of growth, is in the nature of cell expansion. Roughly it might be estimated that half the increase in length of mesocotyls and coleoptiles results from the expansion of cells formed before the resting stage of the embryo.

Although the scutellum may not increase in dry weight with the growing plant, it is an indispensable part of the seedling, without which the embryo could not resume growth. This organ, therefore, should not be disregarded in considering the effect of hybrid vigor on embryo weight. Furthermore, it is quite likely that the weight of the scutellum increases with the resumption of growth after the resting period and that this weight is properly a part of the total dry weight of the seedling, even though it has not resulted from further cell division. The starch of the endosperm is not essential, as excised embryos will grow without it.

Ashby (*2, p. 1010*) gave data for the weights in milligrams of seeds, embryos, scutella, and total embryos for two parents and the F_1 and F_2 of maize, the seed of which was furnished by F. D. Richey. From the table, by subtraction, the endosperm weight can be derived. Taking the mean of the two parents as a base, it is found that the F_1 hybrid exceeds the parents in weight of grain by 14 percent, in weight of embryo by 58 percent, in weight of scutellum by 67 percent, and in weight of endosperm by 8 percent.

From the above data it is evident that the embryonic tissue represented by the scutellum increases in weight over the mean of the parents in the same order as does the embryo. The errors are such that the difference between these two parts of the embryo in percentage increase is not significant.

The relation of increase in weight of embryo to increase in weight of endosperm should throw some light on the nature of hybrid vigor. If dominant favorable growth factors are the sole explanation and dominance is complete, then the embryo and endosperm should show similar increases in weight, as the additional set of genes contributed by the female parent to the endosperm would have no more effect than the single set contributed to the embryo. On the other hand, if the favorable growth factors are not dominant, then the increase in weight of the embryo should be twice that found in the endosperm. This follows if it is assumed that each set of genes in both the endosperm and embryo produces an equal effect on weight, and further, that the genes derived from the two parents produce additive increases. Adding one set to the endosperm having unit effect would result in a 50-percent increase in weight, whereas a similar set added to the embryo would result in a 100-percent increase in weight.

Paddick and Sprague (*10, table 3*) give data that show an almost perfect agreement in the magnitude of percentage increase or decrease in weight of hybrid endosperms and germs. Only two real discrepancies were found, namely, hybrids A 12 ✕ A 55 and A 25 ✕ A 55. In the first of these hybrids the endosperm exceeded the female parent in weight by 34 percent, while the embryo showed only a 2.8 percent increase. In the second hybrid the endosperm exceeded the female parent in weight by 5 percent, while the embryo showed a 27.7 decrease. In their table 2, where 14 paired individual seed comparisons between hybrids and the female parent are shown, there are a number of wide differences between endosperm and embryo in their percentage increase in weight over the female parent. There is only one instance, however, where the hybrid germ shows a greater percentage increase in weight than the endosperm, and this is too small to be of significance.

Brink and Cooper (*6*) found in the case of alfalfa that the cells of the embryo increased in arithmetical progression for the first 6 days after fertilization. These authors also concluded that the rate of increase in number of cells in the embryo was no greater in crosses than in selfs. On the other hand, the number of nuclei in the endosperm during the same 6-day period increased exponentially, and the rate of increase in number was very much greater in crosses than in selfs. The authors point out that under these conditions hybrid embryos are accompanied by more advanced endosperm than inbred embryos. This circumstance might explain the greater size of hybrid embryos at the resting stage if it meant that greater endosperm activity resulted in better nourishment for the embryo. Seven plants were utilized in this study, and, although the crosses were between unrelated plants within the group, it is not clear whether the genetic backgrounds were such that heterosis was to be expected.

MATERIALS AND RESULTS

GERM WEIGHTS OF HYBRIDS AND PARENTS

Some of the seed stocks used by Ashby (*1*) in his original tests were furnished him by the writers and these have been used to check

his results. The cross was between an inbred line of Pipe, self-pollinated for 12 years, and an inbred line of Pawnee, self-pollinated for 20 years. Crossed and self- or sib-pollinated seeds were produced together on the ears of Pipe and separated by means of differences in aleurone color. Two crosses were tested, both having the same Pawnee parent but differing in that in one case the female Pipe parent was self-pollinated whereas in the other the Pipe parent was pollinated with a sib plant. No seed was obtained from the selfed Pawnee male parent. The results are shown in table 1.

TABLE 1.— *Mean weight of whole seeds and endosperm and embryo fractions for single plant pollinations*

SELFED OR SIBBED FEMALE AND COMPARABLE HYBRID SEED ON SAME EARS

Progeny	Air-dry weight of whole seed	Oven-dry weight of—		
		Whole seed	Endosperm	Embryo
	Gram	Gram	Gram	Gram
Pipe 194×Pawnee	0 2467±0 0021	0 2336±0 0022	0 2125±0 0019	0 0211±0.0004
Pipe 194 selfed	2007± .0016	.1882± 0014	.1743± 0015	.0139± 0002
Pipe 195×Pawnee	.2112± 0012	1986± 0013	1812± .0013	0174± 0009
Pipe 195×Pipe 194	.1709± .0089	.1598± .0094	.1481± .0084	0117± .0007

SELFED AND HYBRID SEED ON SEPARATE EARS

Pawnee selfed	0 2120±0 0061	0 1923±0 0055	0 1731±0.0051	0.0192±0 0006
Pipe×Pawnee	2765± .0028	2519± 0026	2291± .0031	0228± 0006
Pipe×Pawnee	2902± 0056	2635± 0049	.2387± 0045	0248± 0008
Pipe selfed	2955± 0031	2662± .0026	.2459± .0029	0203± 0004

Where hybrid seeds are borne on the same ears with selfed seed the increase in weight of endosperm is 22 percent and in that of embryo 52 percent (table 1). These increases also are found where hybrid seed is borne on the same ear with sib-pollinated seed, the endosperm of the hybrid exceeding that of the inbred by 22 percent and the hybrid embryo exceeding the inbred by 49 percent.

Two other hybrids between these inbred lines were examined. In these two cases selfed seed was not borne on the ears with the hybrid seed. The comparison involves three plants of the inbred Pipe, two having been crossed with Pawnee and one self-pollinated. The results are shown in table 1.

Both hybrids had endosperms lighter in weight than that of their female parent, and the embryos were only 12 percent and 22 percent heavier, respectively, than that of the female parent. Grown to maturity, these Pipe × Pawnee hybrids far exceeded their inbred parents in yield.

A set of sister plants was used to test the increase in weight of plants at various periods after planting. These crosses, because of the small number of seeds, could not be used to test both germ weight and growth increase. Seed weights were recorded and are shown in table 2.

TABLE 2.—*Mean weight of whole seeds and estimated weight of embryo for single plant pollinations*

[Selfed female and hybrid seed on same lines]

Progeny	Air-dry weight of whole seed	Esti-mated dry weight of embryo	Progeny	Air-dry weight of whole seed	Esti-mated dry weight of embryo
	Gram	*Gram*		*Gram*	*Gram*
Pipe 181 selfed	0 199±0 003	0. 0136	Pawnee 521 selfed	0 198±0 014	0.0178
Pipe 181 × Pawnee 524	225± 006	.0187	Pipe 182 selfed	.306± .012	.0210
Pawnee 524 selfed	.205± .018	0185	Pipe 182 × Pawnee 523	.313± .022	.0260
Pipe 192 selfed	.220± 008	.0151	Pawnee 523 selfed	.244± .013	.0220
Pipe 192 × Pawnee 521	.258± .011	0214			

From the data presented in table 1, it is seen that the embryos of self- or sib-pollinated Pipe plants are 6.9, 6.8, and 6.9 percent of the air-dry weight of the seed; those of Pipe × Pawnee are 8.6, 8.2, 8.2, and 8.5 percent; and the weight of the embryo of the single Pawnee given is 9 percent of the air-dry seed weight. From other examples not given here of seed weights of sister ears of these lines for this year (1935) it can be safely assumed that these percentages hold reasonably well. They have been used, therefore, to estimate the approximate germ weights of the seeds grown to obtain information on the rate of increase in plant weight. The estimated weights, based on percentages of 6.85, 8.3, and 9, are given in table 2.

These hybrids are identical with those reported by Ashby, though made 4 years later. The parents, therefore, had four generations more inbreeding. The estimated increased weights of hybrid germs over those of the female parent of 37.5, 41.8, and 24.4 percent compare with sister ears actually weighed and presented in table 1 of 52, 49, 12, and 22 percent and with Ashby's (1) material of 18.5 percent.

The field experiment was planted May 20, 1935, in 11 Latin squares, 3×3, planted in three rows, with five seeds 2 inches apart in each hill. The choice of seed and blocks was random. Eliminating the variance due to rows, columns, and means of Latin squares did not improve the accuracy of the test, and since the differences between means of sorts are large, the use of a generalized error derived from the analysis of variance is of doubtful propriety.

The errors, therefore, are estimated from the deviations of the plants of each sort from the mean of that sort for a given sampling date.

The mean weights in grams of the entire plant for the several sampling periods are given in table 3.

TABLE 3.—*Mean oven-dry weight of plants of the inbreds Pipe and Pawnee and the F₁ hybrid between them for various periods*

[Planted May 20, 1935]

Date of sample	Oven-dry weight of entire plant		
	Pipe	Pawnee	F₁ hybrid
	Grams	*Grams*	*Grams*
June 5	0. 145±0.009	0. 177±0 10	0. 274±0.015
June 12	.395± .029	.428± .042	1. 213± .056
June 19	1. 687± .188	2. 126± .287	7. 407± .480
June 26	4. 638± .044	7. 655±1. 451	21. 273±1. 549

The rates of increase for the three periods of 7 days each are given in table 4, and also the significance of the differences in rate of increase between the hybrid and each parent.

TABLE 4.—*Mean rate of increase in dry matter for the inbreds Pipe and Pawnee and the F_1 hybrid between them and differences in rate of increase between hybrid and each parent*

RATE OF INCREASE [1]

Period	Pipe	Pawnee	Hybrid
June 5 to 12	2.73±0.26	2.42±0 27	4.42±0.32
June 12 to 19	4.28± .57	4.96± .83	6.11± .49
June 19 to 26	2 75± .31	3 60± .84	2.87± .28

DIFFERENCES IN RATE OF INCREASE

Period	Hybrid – Pipe	Diff./S.E.	Hybrid – Pawnee	Diff./S.E.
June 5 to 12	1 69±0.41	4.1	2.00±0.42	4.7
June 12 to 19	1 83± 75	2 4	1.15± .96	1.2
June 19 to 26	.12± .42	3	−.73± .89	.8

[1] Ratio of later to earlier value, from data given in table 3.

It will be seen that for the first period (June 5–12) the rate of increase of the hybrid was significantly larger than that of either parent. In the second period, while the rates of increase were all larger than in the first period, perhaps due to better growing conditions, the superiority of the hybrid declined, becoming of somewhat doubtful significance. And in the third period all differences were without significance, the inbred Pawnee actually making a slightly greater increase than the hybrid. The results of this experiment support those of Ashby and Sprague, but it should be noted that the errors of rates are such that differences of less than approximately 30 percent cannot be established. A 30-percent difference in rate would be greater than necessary to produce most of the observed increases in size of hybrids over their parents, irrespective of any differences in initial weight of meristematic tissue.

The data suggest that whatever factors led to the production of heavier embryos before dormancy operated also in the early seedling period.

A second series of F_1 crosses between inbred lines was examined. These lines are wholly unrelated to the Pipe and Pawnee lines and were inbred for only seven generations before the crosses were made. All pollinations were controlled, but each crossed and selfed line involved many plants. The seed was bulked, and 10 samples of 10 seeds each were drawn from the thoroughly mixed stock. The mean weights of various parts of the seeds are shown in table 5.

TABLE 5.—*Mean weight of whole seed and endosperm and embryo fractions for multiple plant pollinations*

[Selfed and hybrid seed on separate ears]

Inbred lines and hybrids	Air-dry weight of entire seed	Oven-dry weight			Weight of embryo [1]
		Entire seed	Endosperm	Embryo	
	Gram	*Gram*	*Gram*	*Gram*	*Percent*
Line A	0.3092±0 0084	0 2772±0 0084	0 2597±0 0084	0.0175±0.0006	6 3
A×B	.3652± .0065	3394± 0058	.3177± .0057	.0217± .0012	6. 4
Line B	.1853± 0037	.1679± .0034	.1555± 0031	.0124± .0005	7 4
C×A	.1984± 0017	1843± 0017	.1738± 0017	.0105± .0004	5 7
Line C	.2180± 0035	1987± 0031	.1822± .0009	.0165± .0007	8 3
Line D	.2530± 0026	.2291± .0023	.2140± .0024	.0151± .0006	6. 6
B×D	.1765± .0058	.1615± .0054	.1486± .0052	.0129± .0039	8.0
D×B	.2903± 0060	.2659± .0058	.2482± .0054	.0177± .0006	6.7

[1] Measured as percentage of dry weight of seed.

As the cross showing the greatest increase in germ weight (A×B) proved to have the highest field yield and that showing no heterosis effect (C×A) proved to have the poorest yield, there is some evidence in this experiment to support the hypothesis that heterosis as exhibited in germ weight will be reflected in field yield. However, it should be noted that all of these lines and crosses are much alike because they were derived from a single variety. This conclusion receives slight confirmation from the field yields of the reciprocal crosses (B×D and D×B), where the cross having the heavier germ had a slightly higher yield.

In the hybrids of Pipe and Pawnee, the greater increases in embryo weights relative to those of endosperm suggest that hybrid vigor is not solely the result of the interaction of complementary dominant factors favorable for growth. However, the data in table 5 support the contrary position. In these hybrids, where increases in weight of embryo were obtained, increases of comparable magnitude were found also in the endosperms. The percentage increase or decrease in weight of embryo and endosperm is shown in table 6.

TABLE 6.—*Percentage increase or decrease in weight of the endosperm and embryo of hybrid seed over that of the female parent and of the average of both parents*

Hybrid between inbred lines	Hybrid—female parent		Hybrid—average of both parents	
	Endo-sperm	Embryo	Endo-sperm	Embryo
	Percent	*Percent*	*Percent*	*Percent*
A × B	22 4	24 0	53. 1	45 7
C × A	−4. 6	−36 4	−21. 3	−38 2
B × D	−4 4	4.0	−19 5	−5 8
D × B	16.0	16 6	34 4	28. 5

The inbred lines are lettered, and it will be noted that hybrids B×D and D×B are reciprocals and that A×B and C×A have one parent in common. It should also be noted that the male parent of hybrid A×B is the female of B×D and the male of D×B.

Hybrids B×D and C×A had germs weighing less than the mean of their parents, and the germs of hybrid C×A actually weighed less than the germs of its female parent. Hybrid B×D does not differ significantly in germ weight from its female parent nor from the mean

of both parents. The differences between the other three hybrids and their parents in germ weights may be considered significant.

When tested with a common check, the crosses A×B and B×D both exceeded the check in pounds of grain per plant, the former by 0.14 pound and the latter by 0.13 pound. Both increases are significant, with probabilities of 0.002. The hybrid C×A, on the other hand, was below the check in yield of grain per plant by 0.09 pound. This decrease may also be considered significant, although the probability of its being a chance decrease is 0.03. The order of these three hybrids in their yields of grain follows that of their germ weights.

Hybrid D×B was not tested, but the following year a direct yield comparison was made between the reciprocals B×D and D×B. In this test D×B exceeded B×D in yield by 4 percent, but the yield test could not establish differences of less than 15 percent. The field yields, however, corresponded with the germ weights.

The results of the field tests of these four hybrids may be considered as at least not contradictory of Ashby's findings. In addition, the germ weights of these crosses confirm Sprague (*12*) and Paddick and Sprague (*10*), in that the weights of hybrid germs are not invariably heavier than those of their parents, although the resulting plants show hybrid vigor.

GERM WEIGHTS IN F₂

The early results on the relation of germ weight to subsequent yield stimulated investigation, and as a result of this enthusiasm, a study was made of the germ weights in the second generation of the hybrid A×B; the F_1 data were given in table 5.

In this study an effort was made to sort the seeds visually into germ sizes irrespective of seed weight. Five groups were made because of pronounced differences in seed shape. There were flat medium, flat large, round medium, round large, and small. The flat and round refer to seed shape; large, medium, and small, to germ size.

The ear proved to have the distribution shown in table 7.

TABLE 7.— *Distribution of the F_2 seeds of the hybrid $A \times B$ based on selection for germ size and seed shape*

Seed character	Seeds	Class		Seed character	Seeds	Class
	Number	*Percent*			*Number*	*Percent*
Flat medium	84	34.3		Small	33	13.5
Flat large	46	18.8				
Round medium	51	20.8		Total	245	100.0
Round large	31	12.6				

Twenty-five seeds from each of these groups were weighed individually for germ size, and the results are shown in table 8.

TABLE 8.— *Mean weight of whole seeds and endosperm and embryo fractions from the F_2 of hybrid $A \times B$*

Seed group	Air-dry weight of entire seed	Oven-dry weight		
		Entire seed	Endosperm	Embryo
	Gram	*Gram*	*Gram*	*Gram*
Flat medium	0.1924±0.0032	0.1774±0.0029	0.1595±0.0027	0.01781±0.00048
Flat large	.2161± .0048	.2009± .0043	.1779± .0038	.02297± .00075
Round medium	.2083± .0082	.1934± .0076	.1737± .0074	.01974± .00064
Round large	.2255± .0071	.2086± .0066	.1866± .0061	.02197± .00100
Small	.1587± .0076	.1462± .0071	.1305± .0065	.01568± .00076

Unquestionably, the selection for germ size was effective, but it will be observed that it was simply an indirect selection for seed size. As a more direct measure of this relation, the correlation between germ weight and the dry weight of seed without the germ has been calculated separately for each of the five seed groups. These five coefficients are shown in the last column of table 9.

TABLE 9.—*Dry weight of germ and correlation with dry weight of seed within five classes in the F_2 of hybrid A × B*

Seed class	Weight of germ [1]	Correlation (r) of germ weight with weight of seed without the germ	Seed class	Weight of germ [1]	Correlation (r) of germ weight with weight of seed without the germ
	Percent			*Percent*	
Flat medium_____	9.3	0.38	Round large_____	9.7	0.43
Flat large _____	10.6	.59	Small_____	9.9	.71
Round medium_____	9.5	.26			

[1] Measured as percentage of air-dry weight of seed.

The subgroup population of only 25 seeds is too small to attach significance to all of these correlations. Combining the five groups into a single population of 125 seeds on the assumption that the selection was not efficient enough to separate the groups, the correlation between seed weight (without the germ) and germ weight is found to be 0.614 with a regression of germ weight on seed weight of 0.083.

It is apparent from table 9 that the relative weight of the germ to the total dry weight (without the germ) does not differ appreciably between the seed groups.

The frequency distribution of germ weight is very irregular, with an ill-defined mode and a mean weight about 10 percent below that of the F_1. The weights ranged from 0.0087 to 0.0324 with a mean of 0.0194. A large part of this unsatisfactory distribution results from the variability of seed weight. When germ weight is corrected for the regression on seed weight, the distribution achieves greater regularity, as shown in figure 1. There is no evidence of bimodality.

In another study, not reported here, germ weights were recorded of Cuzco maize. The mean weight of the oven-dry seeds was 1.149 ±0.021 gm., and the mean germ weight was 0.104±0.003 gm. The germ weight, therefore, was 9.1 percent of the seed weight, which is about the proportion found for most mature seeds.[3]

The correlation of seed weight (without the germ) and germ weight in the Cuzco maize was $r=0.393$ with a regression of germ weight on seed weight of 0.06.

Ashby concluded there was no correlation between seed weight and germ weight because in his material the lines having the heaviest seeds were not the ones with the heaviest germs.

If endosperm weight and germ weight are controlled, at least in part, by the same genic interactions, as would appear to be the case

[3] In immature seeds the dry weight of germs comprises a smaller proportion of the total dry matter. The germs of seed 21 days after fertilization are approximately 6 percent of the total dry weight.

GERMS (MEAN NUMBER)
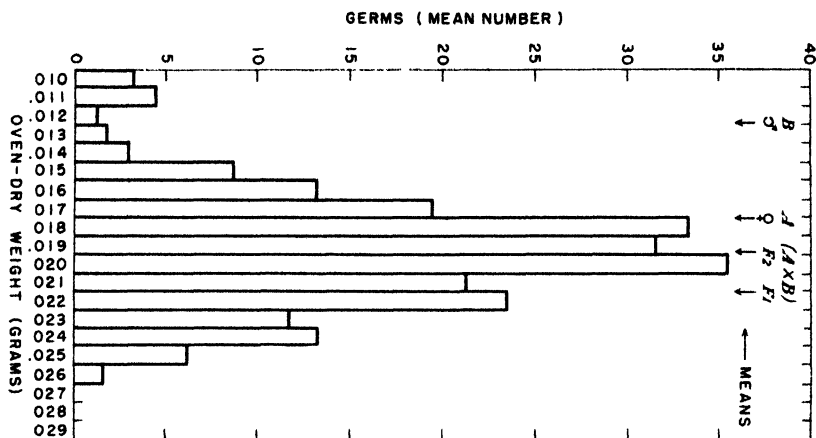
FIGURE 1.— Frequency distribution of germ weight for the seeds of the second generation of the hybrid A ✕ B corrected for the regression of germ weight on seed weight.

from the data shown in table 6, then the weights of these two seed parts would not be independent. It would seem to be a safe inference that when hybrid seeds are heavier than those of their female parent the germs of such seeds will also reflect this greater weight. It is quite likely that interclass correlation would be low where diverse lines comprise the population, and where only a few lines are involved the correlation of seed weight and germ weight might even be negative. In the present examples it is evident that, given a population comprised of a mixture of Cuzco and any of the other sorts considered in this study, the correlation between seed weight and germ weight would be very close indeed.

GERM WEIGHTS IN EARLY STAGES OF GERMINATION

The weight of the germ in the resting stage must be determined by the rate of growth and the period elapsing between fertilization and dormancy. Given germs with equal rates of growth, their final weights must be equal unless their growing periods differ. If the resting stage could be eliminated, as it is in those mutations in maize designated as germinating seeds, or vivipary, the germ weight would not differ in character from seedling or plant weight. It follows that prolonging the growing period of the germ before the resting stage would result in adding to its weight, and since the resting stage can be eliminated it may be argued that when seeds are germinated in the dark, following their dormancy, the rate of increase in seedling weight with time corresponds to that which would have obtained had no resting period intervened. If this holds, then hybrids with small germs in the dormant stage should have slower growth rates in the early stages of germination than hybrids with heavy germs.

As a preliminary test of this point, three lots of seed from two of the F_1 hybrids shown in table 5, namely C✕A and D✕B, were germinated in the dark under identical conditions, and samples were withdrawn at the end of 24, 48, and 72 hours. From these samples the results given in table 10 were obtained.

TABLE 10.— *Mean oven-dry weight per seed of maize after various periods in a germinating chamber*

[F₁ hybrids (C × A) and (D × B) shown in table 7]

Period	Hybrid C × A			Hybrid D × B		
	Entire seed	Endosperm	Embryo	Entire seed	Endosperm	Embryo
	Gram	*Gram*	*Gram*	*Gram*	*Gram*	*Gram*
Resting	0 1843±0 0017	0 1738±0 0017	0 01053±0 00038	0 2659±0 0058	0 2482±0 0054	0 01764±0 00057
24 hours	.1792± 0026	.1618± .0024	01738± .00011	2635± .0057	.2384± .0053	02494± .00051
48 hours	.1798± .0024	1618± 0022	01810± .00028	2762± .0061	.2500± 0056	.02619± 00086
72 hours	.1827± .0031	.1615± .0027	02116± .00046	.2628± .0041	.2344± .0037	02837± .00094

Considering the rate of increase in oven-dry weight of embryo, it is seen that C×A had a rate of 1.22 and D×B a rate of 1.17. The rates for the several periods are given in table 11.

TABLE 11.—*Mean rate of increase in dry matter of the germs of germinating seeds of the hybrids C×A and D×B for various periods*

Period	Hybrid C×A	Hybrid D×B
1st 24 hours	1 65±0 06	1 41±0 05
2d 24 hours	1 04± 02	1 05± 04
3d 24 hours	1 17± .03	1 05± .05
Entire period	1. 22± 10	1 17± 07

It is seen that the hybrid C × A, which started with the less capital, had the more rapid rate of increase. The high rate in the first 24 hours accounts for most of this greater rate. If it is assumed that the germs of both hybrids were potentially of the same weight and that the observed difference in dormant weight resulted from an interrupted development prior to maturity in the case of C × A, then the rate of increase of the two hybrid germs might be compared for the periods when their initial weights were identical.

After 24 hours in the germinator, hybrid C × A had germs comparable in weight to those of hybrid D × B at the resting stage. The rate of increase for C × A for the period after the first 24 hours is 1.10.

None of these differences in rate, except that for the first 24-hour period, may be considered as significant.

RATE OF INCREASE IN WEIGHT OF GROWING GERMS

No data were collected showing the rates of growth of the embryos of these hybrids before dormancy. However, field studies have been made on other samples. The determination of rates of weight increase in germs on plants growing under field conditions offers many pitfalls because of the changing environment.

Two sorts of tests have been made with seeds maturing in the field. In one test, sections from ears pollinated at a known time were removed at various periods and rapidly dried. The remaining sections were removed at later periods, and the differences in germ weight between the periods were used to determine the rate of growth. In another set of tests, groups of whole ears pollinated on the same day were harvested at different dates, and the differences in germ size

between the early and later periods were used to determine the rate of growth. In both experiments the ears were of the same stock and the pollen used was composited from a number of sister plants.

The first method introduces a possible error if the seeds at the top half of an ear have smaller germs than those at the bottom half. The error from this source may be reduced by using for germ weights the seeds nearest the cut half in each case. However, for reasonable populations of germs, several rings of seeds must be used, so that even under ideal conditions many of the seeds on the separate ear sections are widely separated. Further, it often happens that on the section of the ear left to mature on the plant the seeds near the cut end either fail to attain a dormant stage or else this is of short duration, as many of them germinate before harvest.

The second method, as well as the first, introduces the unknown element of environment, as conditions for germ growth may change so that the observed differences may result, at least partly, from this cause. In the second method also there is introduced the element of genetic variation between plants as well as any differences of environment resulting from the fact that the plants occupy different locations.

The results obtained by these two methods are shown in table 12.

TABLE 12. *Mean daily rates of increase in germ weights of seeds developing on field-grown plants*

Part of ear and period on which rates were based	Daily rates of increase in germ weights of seeds on--		Part of ear and period on which rates were based	Daily rates of increase in germ weights of seeds on	
	Ears pollinated July 27	Ears pollinated August 1		Ears pollinated July 27	Ears pollinated August 1
Ear sections			Whole ears		
Aug. 21 to 23	1 135±0 039	1 327±0.090	Aug 23 to 26	1 004±0 029	1 084±0 047
Aug. 23 to 26	1 072± .039	1 064± 021	Aug. 26 to 28	1 162± 025	1 192± 059
Aug 26 to 28	1 082± 081	1 163± 046			
Aug. 28 to 30	1 056± .011	1 059± 041			

The errors show how unsatisfactory these methods are. There is little agreement between the results obtained on whole ears and on sections where the pollinations were made on July 27, whereas the agreement between these two methods is very good for the pollinations made on August 1. The study would have to be greatly extended before it could be determined whether or not the rate of increase in weight at these early stages was constant.

In conclusion, it may be pointed out that these early rates are of the same order of magnitude as those found for the first 3 days of germination.

GERM WEIGHTS OF VARIOUS SEED TYPES

On the theory that onset of dormancy interrupts the growth of the embryo it was thought possible that where sweet and starchy seeds developed upon the same ears the sweet seeds would have heavier embryos than starchy seeds, because sweet kernels retain a high moisture percentage longer than starchy seeds.

A self-pollinated ear segregating for sweet from a progeny repeatedly backcrossed to starchy until 93.75 percent of the genes were

common to both seed types was examined. This ear gave the weights shown in table 13, ear 1.

TABLE 13.—*Mean weight of whole seeds of various types, and endosperm and embryo fractions*

[Seeds of contrasting types borne on same ear]

Ear No. and seed type	Air-dry weight of whole seed	Oven-dry weight of whole seed	Oven-dry weight of endosperm	Oven-dry weight of embryo
	Gram	*Gram*	*Gram*	*Gram*
Ear 1:				
Starchy	0 3153±0 0052	0 2929±0.0049	0 2788±0 0051	0 01409±0 00051
Sweet	.2569± .0057	2389± .0063	2065± .0068	03240± .00195
Ear 2				
Starchy	4691± .0085	4333± 0080	.3888± .0072	.04449± 00128
Sweet	.3368± 0099	3084± .0089	.2728± 0087	03558± 00120
Ear 3				
Horny	.3257± .0039	.3045± 0038	2799± .0036	.02456± .00064
Waxy	.3264± .0045	.3040± .0042	.2807+ 0043	.02333± .00081
Ear 4:				
Horny	.3533± .0030	.3308± .0030	.3057± 0032	.02505± .00079
Waxy	.3377± .0037	.3147± .0034	.2929± .0035	.02181± .00067
Ear 5:				
Flinty	.2508± .0021	.2374± .0022	.2177± 0022	.01964± 00064
Floury	.2479± .0023	.2315± 0024	.2094± .0196	02221± .00066
Ear 6:				
Blue	.2631± 0021	2409± .0016	.2197± 0021	02118± .00098
Red	.2678± .0042	2452± .0038	.2178± 0042	02740± 00076
Ear 7:				
Yellow	1849± .0013	.1726± .0011	.1534± 0011	01927± 00025
White	.1812± 0018	.1684± .0017	.1506± .0018	01777± 00033

The difference in weight of embryo is clearly significant and suggests that the slower maturation of sweet kernels, as indicated by their higher moisture content, may afford the germ a longer growing period before dormancy.

A second ear was examined from a similar but unrelated backcrossing experiment where 87.5 percent of the genes were common to both seed types. In this instance the seeds did not result from self-fertilization but were from crosses between sibs. The weights are given in table 13, ear 2.

In this instance the embryos of sweet seeds are most certainly lighter in weight than those of starchy seeds on the same ear, a result which contradicts the hypothesis of a longer growing period for the embryos of sweet seeds.

A similar study was made with respect to germ weights in horny and waxy seeds on the same ears where because of repeated backcrossing most of the genes were common to both seed types. The weights are given in table 13, ears 3 and 4.

Insofar as these two ears are useful for generalization, it appears that when the female gametes are all waxy and the male is heterozygous (ear 4) the germs of nonwaxy seeds are significantly heavier than those of their waxy sibs. The difference in weight of germ is greater than the corresponding difference in weight of endosperm. When the female is heterozygous (ear 3), the endosperm of the waxy seeds is actually, though not significantly, heavier than that of the horny seeds on the same ear. The germs of the waxy seeds, however, are lighter in weight, though the difference of 5 percent is not significant.

There is no evidence from either of these ears that the waxy type of endosperm provides a longer growing period for the germ before the onset of the resting stage.

A fifth ear was examined, where the contrasting seed characters were flinty-floury. This ear was from an Indian variety inbred for only two generations. The observed weights are given in table 13, ear 5.

This result might be taken as an indication that the physical character of the endosperm affected germ growth. However, two other ears were tested where the differences in seed type were colors and the endosperms were alike in texture.

The first of these is an Indian corn inbred for several generations but segregating for blue and red aleurone (*Pr, pr*). The observed weights are given in table 13, ear 6. In this case the embryo weight of the seeds having the recessive color class exceeds that of the dominant seed group by almost 30 percent.

The second of these color groups involved yellow and white endosperms. The observed weights are given in table 13, ear 7. In this instance the difference in weight of embryo between the two seed classes is also significant, but the recessive seed class has the lightest embryos.

Although there is little likelihood that heterosis is involved, the differences in germ weight between seed classes of the same ear are of the same order as those found between hybrids and their inbred parents and in some instances are of such a magnitude that they should result in wide differences in mature plant size, unless there is a compensating difference in growth rate.

From these data, accumulated for the several seed classes paired on the same ears, it is not possible to formulate conclusions other than that significant differences in germ weight are to be found in various seed groupings, even where most of the gene background is uniform for the classes.

SUMMARY AND CONCLUSIONS

The results reported by Sprague (*12*) are confirmed. Hybrid vigor is often, though not invariably, reflected in the dry weights of the resting embryos of maize. However, in those cases where the hybrid embryos are lighter in weight than those of their parents the plants grown from them exceed the parents in size.

The rate of increase in dry matter following dormancy is greater in hybrids than in their parents in the early stages but the greater rate is not maintained to maturity.

Seed groupings based on endosperm texture and color but with uniform genic backgrounds disclose differences in germ weights.

The increase in weight of embryo relative to the increase in weight of endosperm offers equivocal evidence on the hypothesis that hybrid vigor results from the interaction of complementary dominant factors favorable for growth.

The possibility remains that the measure of heterosis afforded by the germ weight of hybrids, in comparison with that of their parents, can be used as a preliminary evaluation of a series of crosses. The data available at present are inadequate to determine just how useful this criterion may be and, of course, the dry weight of the ultimate plant is only one element governing the choice of hybrids. If germ weights prove of value, the tedious operation of removing dormant germs apparently can be obviated by utilizing the early stages of germination.

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RELATION OF NAVAL STORES YIELDS TO FREQUENCY OF CHIPPING [1]

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INTRODUCTION

The common commercial practice in wounding, or chipping, long-leaf pine (*Pinus palustris*) and slash pine (*P. caribaea*) to obtain gum from which naval stores are distilled, is to make the wounds, or streaks, at weekly intervals from March to November, continuing from season to season until the series of streaks, or face, is run up to a practical working limit of about 9 feet.

Early in the history of the industry, operators learned that the yield of gum so obtained is in some way related to the weather, particularly to the temperature, and that the yield is much less in the cooler winter months than in the warmer summer ones. Some of the more progressive producers observed that fair gum yields could be obtained by chipping during periods of warm weather in the winter. A few operators, in more recent years, have made a practice of double chipping, that is, chipping twice a week during the summer. These variations from customary weekly and seasonal chipping practice are used mainly to increase the total production per season or year. Some of the winter chipping, however, is done to prevent the formation of unproductive pitch-soaked wood, which forms when a turpentined tree remains unchipped for several months.

There are other reasons for departing from customary chipping schedules. If an operator finds it desirable for various reasons to lengthen or shorten the utilization period, he chips less or more than the customary number of streaks per season, thereby reducing production per season and distributing it over more seasons or increasing production over fewer seasons. This calls for a somewhat flexible labor supply as well as availability of woods work other than turpentining. Labor conditions, in fact, are often responsible for changes in chipping schedules. In times of labor scarcity, streaks will be missed during the regular season, whereas a plentiful supply of labor often results in more frequent chipping than usual, especially during the hottest months. Chipping frequency thus obviously depends, in part at least, upon economic factors. Again, the importance of considering silvicultural and forest-management objectives is becoming apparent, and these factors are already beginning to overshadow the economic factors. In thick stands of timber, for example, the trees least desirable for the final crop can be chipped frequently for quick naval stores exploitation prior to thinning. Likewise, it is more profitable to exploit saw timber quickly for gum prior to logging than to prolong the turpentining and expose such timber for several more years to the risks of fire, wind, and insects.

Because the reasons for employing different chipping-frequency schedules are so numerous and varied, there has been in recent years considerable investigation of the effect of frequency of chipping on gum yield. In Germany, such names as Münch, Schierlinger, and Cieslar are primarily associated with these investigations; in France, Oudin; in Sumatra, Buys; in Russia, Ivanov; in Spain, Iturralde; and in the United States, Shorger, Herty, and Wyman. Detailed information, however, on the relations between weather and gum flow is for the most part lacking. The comprehensive basic study of Harper and Wyman [2] on this relationship formed the first thorough basis for evaluating the effect of chipping frequency on yield. They state:

> The study has afforded a basis for estimating, for any given average air temperature, the gum yield to be expected from a streak and the optimum interval between chippings. In general, the temperatures of the months of November to March, inclusive, are found suitable for semimonthly chipping, those of July and August for semiweekly chipping, and those of the remaining months for weekly chipping, for longleaf pine. A similar schedule is applicable to slash pine, but for this species the optimum chipping intervals are in general longer than for longleaf pine.

Because they were working almost entirely with weekly chipping, howeve , they were not able to determine how long or how much gum a streak will produce if allowed to run until it ceases entirely. Furthermore, although they demonstrated conclusively that season or temperature must be the deciding factor in varying the interval between chippings, they did not determine actual yields or the effect of frequent and continuous chipping on the vitality of the timber.

Recognizing the importance of chipping frequency and the determination of optimum intervals between chippings, the Southern Forest Experiment Station started in the spring of 1934 on the Olustee Experimental Forest in northeast Florida an experiment to determine for longleaf pine (1) the effect of different frequencies of chipping on the yield capacity of a tree, and (2) the comparative total yields of gum from varying frequencies of chipping. At the same time another study was begun to determine for both longleaf and slash pine (1) the total yield of gum per streak for different months of the year when the streak is allowed sufficient time to run dry, that is, to cease yielding gum, and (2) the time required for a streak to cease yielding gum in different months.

Details of the second study have been reported briefly elsewhere.[3] They will not be recounted here, but the summarized results will be combined herein with the results of the first study in discussing optimum intervals between chippings.

FIELD WORK

In establishing the frequency-of-chipping study, 66 trees were selected, on the basis of diameter at breast height [4] and growth rate (number of rings in last radial inch), from a rather uniform, well-stocked pure stand of round second-growth longleaf pine approximately 35 years old. The trees then received the first streak about 7 inches from the ground. Galvanized tins and flat-bottom cups

[2] HARPER, V. L., and WYMAN, LENTHALL. VARIATIONS IN NAVAL-STORES YIELDS ASSOCIATED WITH WEATHER AND SPECIFIC DAYS BETWEEN CHIPPINGS. U. S. Dept. Agr. Tech. Bul. 510, 35 pp., illus. 1936.
[3] LIEFELD, T. A. HOW LONG WILL A STREAK YIELD GUM? Naval Stores Rev. 48 (50): 10, 14, illus. 1939.
———— THE EFFECT OF SEASON ON STREAK BEHAVIOR. Naval Stores Rev. 49 (3): 14. 1939.
[4] Measured 4½ feet above ground.

were installed in shallow broadax incisions.   Streaks ½ inch high and
½ inch deep were chipped on schedule throughout the duration of the
experiment by the regular chipper assigned to the experimental forest.

During a preliminary period, April 9 to May 29, 1934, 8 streaks
were chipped on all the trees at weekly intervals.   Total yield per
streak was recorded for each tree to obtain an indication of individual
yield capacities.   The trees were then assigned permanent numbers
and grouped into 6 treatment groups of approximately equal potential
yield.   Each group consisted of 10 trees and 1 extra to replace any
which might be killed or weakened by agencies other than those
studied.

The three chipping schedules shown in table 1 constituted the
experimental treatments, which were begun June 4, 1934.   Treatment
1 represented the customary seasonal chipping practice.   Treatment 2
was slightly heavier; the intervals between chippings were varied
according to seasons, and winter chipping was included.   Treatment 3
was considerably heavier than 2 during the summer period.   When
the treatments were selected, it was anticipated that treatment 1
would be the check by which the others would be compared; that
treatment 2 would represent the optimum in the way of a varying
frequency schedule; and that treatment 3 would be too frequent for
sustained yield.

The 66 trees selected permitted 2 replications of each treatment of 10
trees.   One group within each treatment was treated on a certain day
and the other group on the following day, in order to obtain more
observations of air temperature.   Chipping and gum-weighing
schedules were constantly posted so that the plans of the study could
be adhered to rigidly.   Field inspections were made every week and
notes taken that might be of value in subsequent office analyses.

TABLE 1. - *Number of streaks chipped monthly at regular intervals, in three
experimental treatments* [1]

Month	Treatment 1	Treatment 2	Treatment 3
January	0	2	
February	0	2	2
March	2	2	4
April	4	4	4
May	4	4	8
June	4	4	8
July	4	8	12
August	4	8	12
September	4	4	8
October	4	4	4
November	2	2	2
December	0	2	2
Total number of streaks	32	46	66

[1] Treatments 1, 2, and 3 represent respectively common practice, medium work, and heavy chipping.
Figures given are for 4-week months and hence only approximate actual chipping, which is by calendar
weeks.

The 24-hour yield collected in the cups and the total yield for each
streak were weighed, whenever the schedule called for a weighing,
between 7:30 and 9:30 a. m., on a Harvard balance scale carried
from tree to tree.   A transparent hood effectively shielded the scales
from excessive air movements.   Before the cups were weighed,
however, the scrape, or hardened gum adhering to the face, was
added to the cups.   At the same time, as much as possible of the trash

and rain water present was carefully removed or poured off. This field procedure of chipping and weighing according to schedule continued for the duration of the study.

At the end of December 1936, a total of 206 streaks had been applied in treatment 3 and a height of approximately 9 feet had been reached. These faces were considered worked out and were discontinued. Those in the other treatments, however, were still operable; only 92 streaks had been chipped in treatment 1, and 143 in treatment 2. It was decided to work out the first or front faces of treatment 2 with a total of 206 streaks, at which time it would be abandoned entirely. This happened early in May 1938. Chipping was discontinued on the front faces of treatment 1 at the same time as in treatment 3, even though 114 additional streaks, or nearly 4 more years of treatment, could have been applied. It was deemed more desirable to begin simultaneously a second or back face in both treatments 1 and 3 early in 1937, in order to provide data for a comparison of back-face yields in treatments 1 and 3. Front-face yields from treatment 1 were extrapolated from the time the faces were discontinued until they would have been worked out. Cups were installed for back faces in treatments 1 and 3 on March 29 and April 5, 1937, respectively. These dates also marked the beginning of the treatments. On October 24, 1938, the sixty-fourth streak, or the last of two full seasons, was chipped in treatment 1. Chipping continued in treatment 3 for a total of 137 streaks until the end of the year, when all field work was discontinued.

ANALYSES OF DATA

Two separate analyses of the data were made, one to determine the effect of the three treatments on the yield trends of the groups, and the other to compare the actual yields from the treatments. The variables, selected as being primarily important in such a study as this, were the same in both analyses but expressed in different terms. Gum yield of a 10-tree group, in whole grams, was the dependent variable (X_1). In the first analysis it was the yield recorded for the first 24 hours after chipping; in the second analysis it was the total yield per streak. The independent variables were temperature (X_2) and time (X_3). The temperature, which in both analyses was expressed as maximum air temperature (in degrees Fahrenheit) that occurred on each day of chipping, was obtained from the daily thermometer and thermograph readings at a standard Weather Bureau shelter on the experimental forest, about 1,000 feet from the scene of the study. Time in the first analysis was expressed as the number of days or weeks elapsed since treatments began, and in the second analysis as the number of the streak.

Gum yields in the first 24 hours were found to be no more effective than total yield per streak in expressing the effect of treatment on the vitality or yield capacity of a 10-tree group. Only the final results, therefore, of the first analysis are presented in figure 1, showing for the different treatments the net trend of yield (effect of temperature largely removed) since treatment began, or the effect of treatment on yield capacity. The solid, dashed, and dot-dashed portions of the curves are actual trends computed from the data, whereas the dotted portions are extrapolations up to the time the faces would have been

worked out if an equal number of streaks had been applied in all
treatments.   Approximately half the front-face trend of treatment 1
has been extrapolated.

In the second analysis—comparison of the yields from the different
treatments—interest centers around the partial regressions of yield on
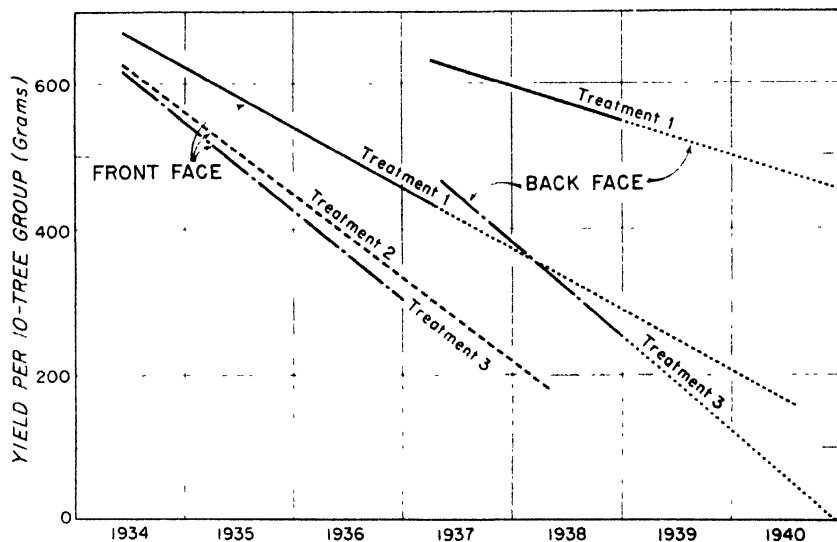
FIGURE 1.   Results of the 24-hour gum-yield trends of treatments 1, 2, and 3.
The dotted portions of the curves are extrapolations as explained in the text.

time, expressed as number of streak, with the effect of temperature
again largely removed.   Multiple linear regressions of yield on tem-
perature and number of streak in the form,

$$X_1 = a_{1\,23} + b_{12\,3}X_2 + b_{13.2}X_3,$$

were computed separately by the least-squares method [5] for front and
back faces and also for each treatment.   First approximations are
shown in table 2.   Curving these values by successive approximations

TABLE 2.- *Multiple linear regressions and other pertinent data for curves showing
trend of total yield per streak*

Face and treatment No	Regression	Means [1]			Number of observations	Multiple correlation coefficient
		X_1	X_2	X_3		
Front face.						
1	$X_1 = 13\,226X_2 - 1.439X_4 - 205\,277$	882.330	87.415	47.602	176	0.489
2	$X_1 = 2\,704X_2 - 2.603X_3 + 779.456$	734.274	84.240	104.859	405	.670
3	$X_1 = 3.701X_2 - 1.657_3 + 555.593$	700.121	86.616	106 259	398	.564
Back face						
1	$X_1 = 11.630X_2 + 3.184X_3 - 158.324$	948.359	86.258	32.500	128	.710
3	$X_1 = .605X_2 - .901X_3 + 634\,229$	624.179	86.179	69.048	273	.256

[1] X_1 = Total gum yield per streak per 10-tree group, in grams; X_2 = maximum air temperature, on day of
chipping, in degrees Fahrenheit; X_3 = streak number.

[5] EZEKIEL, M. METHODS OF CORRELATION ANALYSIS. 427 pp., illus. New York and London 1930.

allowed adequately for temperature and at the same time evolved curvilinear partial regressions of yield on streak number which would follow the actual data more closely than the linear (fig. 2).

In figure 3 the partial regressions of yield on temperature are shown as corrections to the regressions of figure 2. Values were then read from the partial regressions of yield on streak number to form curves of cumulative yield over streak number. These cumulative-yield curves were superimposed for easier comparison, as shown in figure 4, *A*. The same data presented on the basis of calendar time rather than streak number are given in figure 4, *B*.

DISCUSSION

Whenever gum yield from such chipping specifications as were used in this experiment is plotted over a time interval of 3 or more years, a definite downward trend will always be observed. To a small extent this is due to the gradual decrease in face width as chipping progresses up the tree to conform with the natural taper of the stem, but primarily to a gradual decrease in vigor or yield capacity of the tree. This decrease varies, of course, with the intensity of the turpentining, a phenomenon which has been observed and measured repeatedly in numerous naval stores studies, but both face taper and decline in vigor affect trends more markedly after the third year. It is to be expected, as verified by the slopes of the curves in figure 1, that the rate of decline would be greatest for treatment 3 (both the front- and back-face work) and least for treatment 1. Had treatment 1 continued until the faces were worked out, its downward trend undoubtedly would have been greater.

The back-face trends of treatments 1 and 3 reflect somewhat the same situation. Had the front faces of treatment 1 been worked out by continuing the treatment through 1940, before installing back faces, the rate of decline of back-face trends probably would have been greater and the general level somewhat lower. It should be pointed out that the general levels of the front-face trends are relatively unimportant. That they are not the same merely indicates that the preliminary 8-week chipping period was not sufficiently long to establish reliable yield capacities for the individual trees in the study, and that the assignment of trees into groups of equal yield capacities was not entirely successful. The slopes of these trend curves, however, are of major importance in that they portray the drain in vigor resulting from the different treatments. In the back-face curves, both the levels and the slopes are important, and would have been even more so if the front faces of treatment 1 had been worked out prior to back-facing. It is apparent, nevertheless, from the rapid rate of decline of treatment 3 during the period of back-facing, that timber which is to be chipped with the frequency of treatment 3, and probably even that of treatment 2, should be allowed to rest for at least 1 year before back-facing is begun. There does not appear to be sufficient difference in the effects of treatments 2 and 3 on the yield trends to prohibit, on the basis of decrease in vigor alone, such frequent chipping as in treatment 3.

Figure 4, *A*, in which readings from figure 2 have been converted into cumulative yields and plotted over streak number, shows that when comparisons of total production from front faces are made, and when the same total number of streaks has been chipped for each
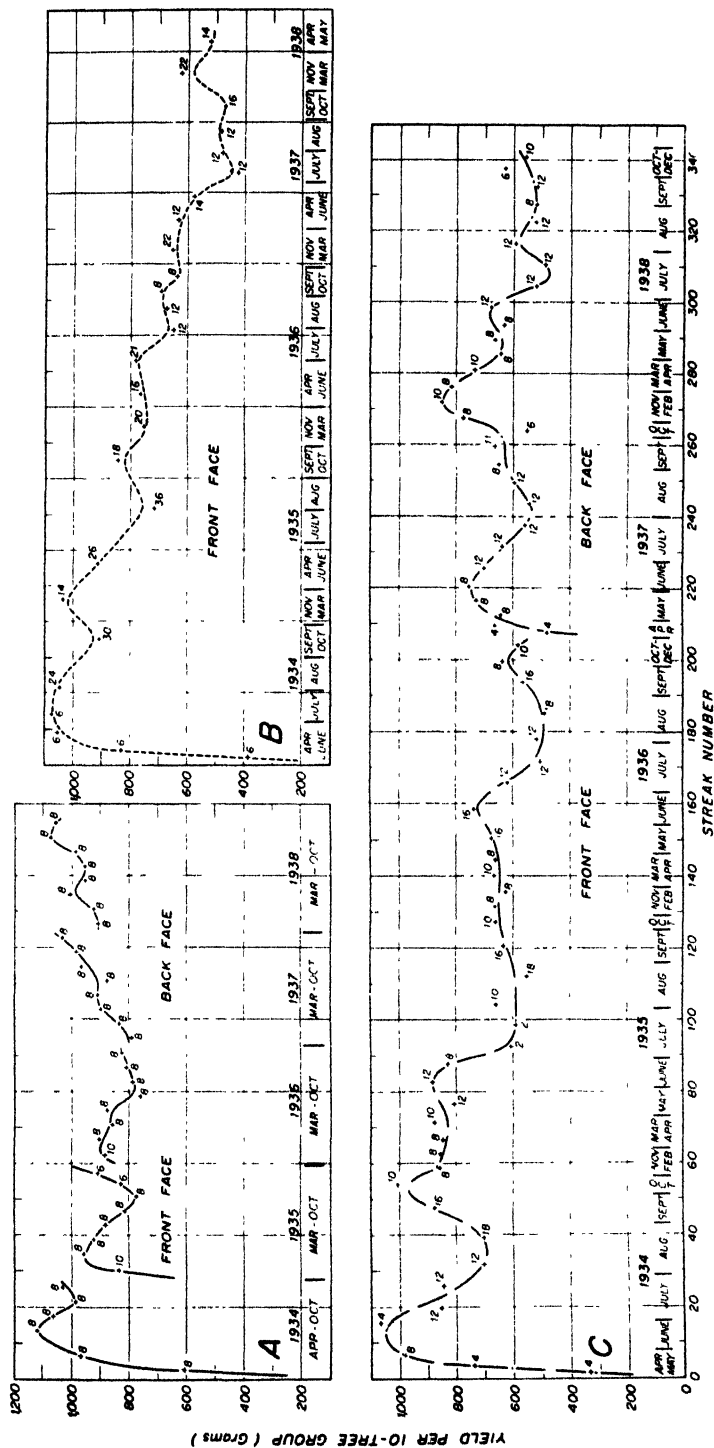
FIGURE 2.—Partial curvilinear regressions of total gum yield per streak on streak number: *A*, Treatment 1; *B*, treatment 2; *C*, treatment 3.
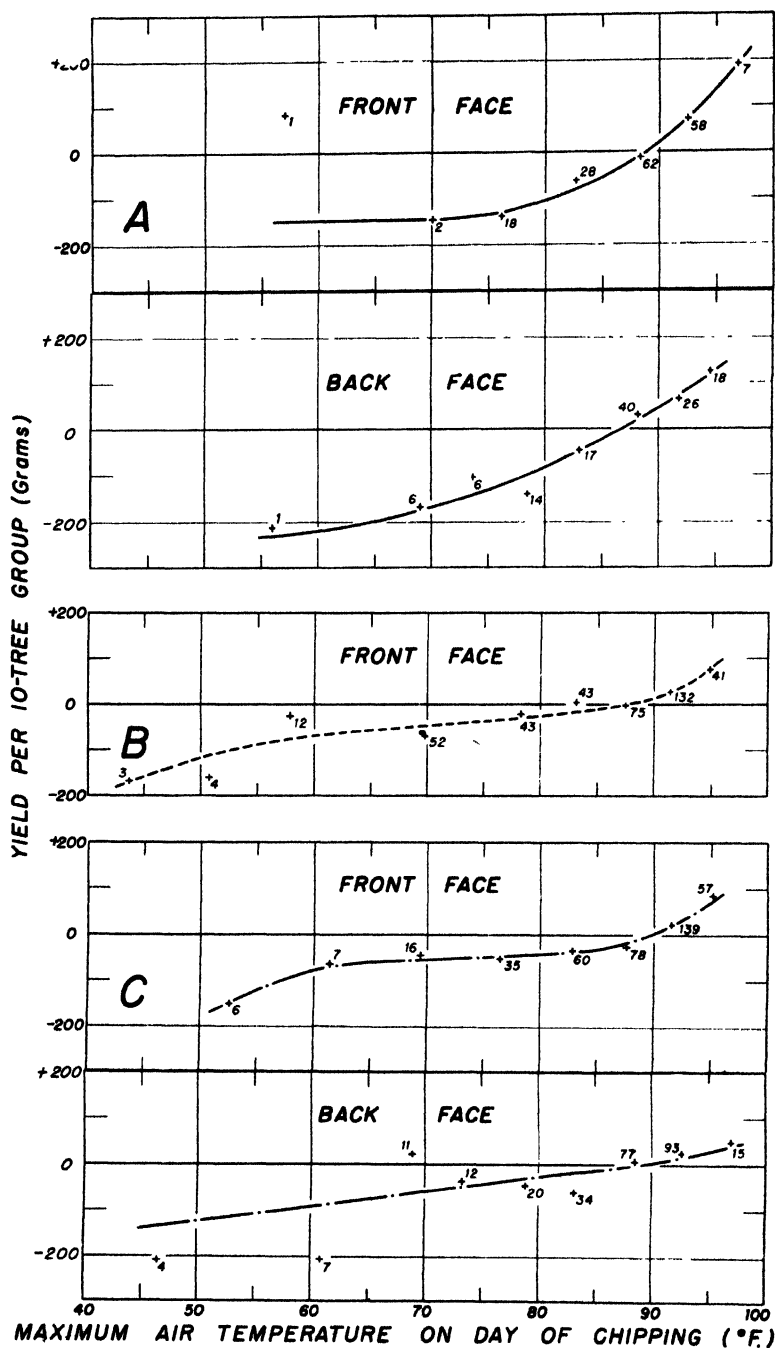
FIGURE 3.—Partial curvilinear regressions of total gum yield per streak on temperature, as corrections to figure 2: *A*, Treatment 1; *B*, treatment 2; *C*, treatment 3.

treatment, treatment 3 is approximately 10 percent inferior to treatment 1 and about 5 percent inferior to treatment 2. Treatment 2 is slightly superior to treatment 1, except possibly for the last year of work, i.e., after about streak No. 160. This superiority of treatment 2 is problematical, however, since the cumulative-yield curve of treatment 1 had to be extrapolated after streak No. 92. Obviously such frequent chipping as in treatment 3 hardly would be expected to surpass less frequent work, when comparisons are made on the
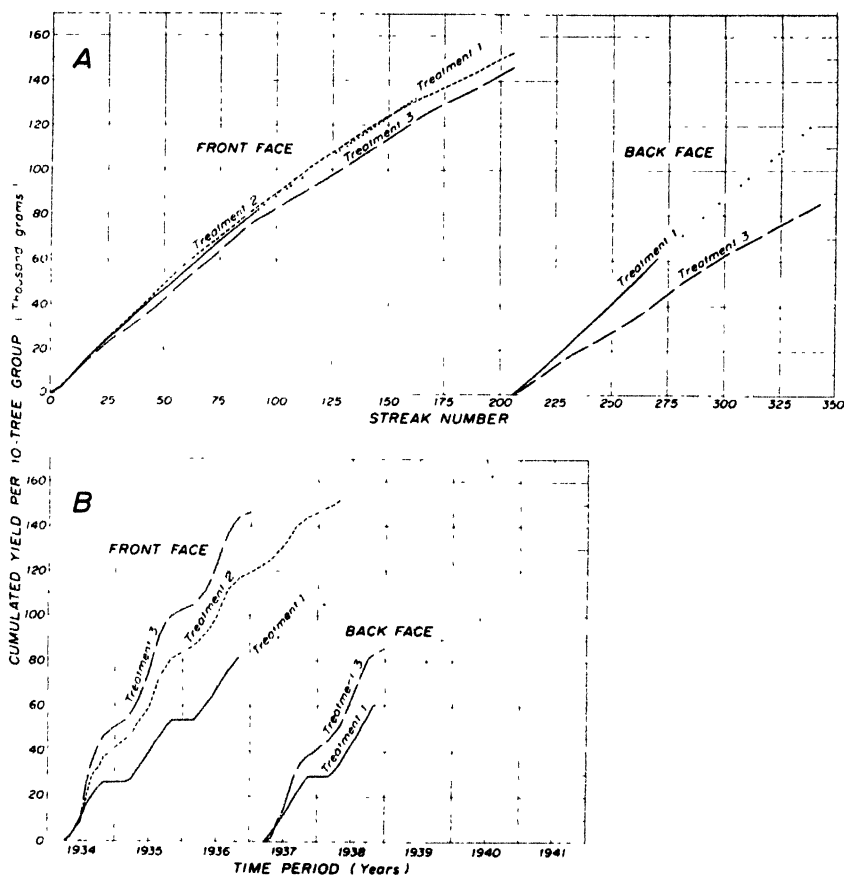
FIGURE 4.— Comparison of total gum yield per streak trends of treatments 1, 2, and 3: *A*, On the basis of streak number; *B*, on the basis of year of work.

basis of total yield per streak or cumulative yield, because the time during which most of the streaks are allowed to flow is diminished. The advantage of frequent chipping, for certain objectives at least, lies in greater production during a given length of time or, conversely, a shorter period of time to produce a given amount of gum. This advantage in time is clearly demonstrated in figure 4, *B*, although it is evident that total production from a face is diminished somewhat by heavier work.

In determining the effectiveness of any chipping-frequency schedule,

information is needed as to the rate and length of time a streak chipped at different seasons of the year will yield gum when allowed sufficient
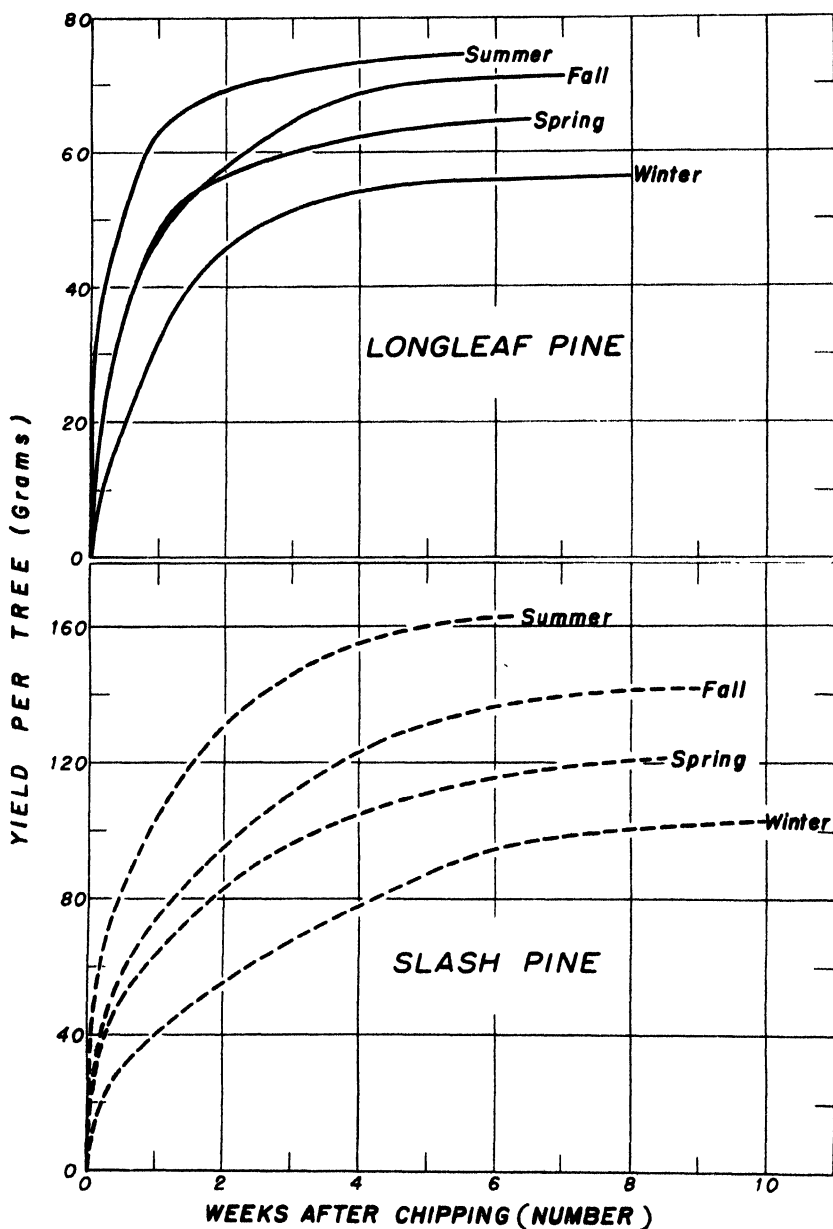
FIGURE 5.—Cumulative gum-yield curves for streaks chipped at different seasons of the year and allowed to run dry.

time to cease yielding entirely. Data on total yield per streak, which although not representative are at least indicative, are presented in figure 5 and table 3 for both longleaf and slash pine, as supplied by

the second study already mentioned. Values read from figure 5 at abscissa-axis values 1, 2, 3, and 4 are the basis for table 3.

TABLE 3.—*Relative gum yields from streaks chipped at different seasons of the year and allowed to flow for 4 weeks*

Species and season of streaking	First week —ratio to first week of summer	Ratio of other weeks to first week		
		Second week	Third week	Fourth week
Longleaf pine:	*Percent*	*Percent*	*Percent*	*Percent*
Summer	100	9	4	3
Spring	77	16	7	6
Fall	73	22	15	9
Winter	50	44	17	8
Slash pine:				
Summer	100	26	15	9
Spring	60	36	21	15
Fall	69	33	19	18
Winter	37	40	30	27

When the study was started it was anticipated that the rate of gum yield would be practically the same from frequent as from infrequent chipping, thus yielding appropriate data for detailed comparisons of different frequency schedules. Data from the study showed, however, that gum flow is more sluggish when the streaks are chipped at long intervals than when chipped weekly or more frequently. The curves of figure 5, based on a chipping interval of 6 to 10 weeks, do not portray the more rapid rise of curves based on weekly chipping. Nevertheless, a few general conclusions can be drawn from the figure. It will be noted from the termini of the individual curves that slash pine consistently flows somewhat longer than longleaf pine and that for both species the time required for a streak to cease yielding gum is greatest in winter (December 22 to March 21) and progressively less in fall (September 22 to December 21), spring (March 22 to June 21), and summer (June 22 to September 21).

The shapes of the curves show that, after the second or third week, yields drop off more rapidly in hot than in cold weather. Evidently weekly chipping during the winter would be poor practice because too much potential yield would be lost from each streak. On the other hand, it would be equally poor practice to permit a streak to run for 6 or 8 weeks in the summer because too small a production to be profitable would result.

SUMMARY AND CONCLUSIONS

In this study of the effects of different frequencies of chipping on the yield trends and total yields of longleaf pine turpentine timber, three treatments were applied, approximating customary seasonal chipping practice, moderate year-round practice, and heavy year-round practice. The gum yield during 24 hours and the total yield per streak were recorded from two 10-tree groups in each treatment, for approximately 4 years, together with the maximum air temperature on the days of chipping.

Comparisons of the partial linear regressions of yield on time, after allowing for a curvilinear effect of temperature, brought out the fact

that neither moderate nor heavy year-round chipping causes excessive drain on the vitality of the turpentined timber.

Comparisons of cumulative-yield trends compiled from curvilinear partial regressions of yield on time, after allowing for a curvilinear effect of temperature, made clear the advantages of frequent chipping when rapid exploitation and high annual or seasonal production are desired. It was evident, however, that chipping frequencies should not exceed three times a week during the summer nor every other week during the winter.

Where long working life of the same faces or trees is considered more important than high production per year, the study points to the schedule of semiweekly chipping in the summer, weekly in the spring and fall, and semimonthly in the winter as recommended in 1936 by Harper and Wyman. Even in the most conservative work, however, loss of gum yield would result if streaks were chipped less frequently than every 2 weeks in the summer and every 6 to 8 weeks in the winter.

If for any reason varying intervals between chippings are out of the question, because of conditions existing in an operation, the results obtained would indicate the desirability of weekly chipping from March to November and chipping every other week during the winter, or, for longer working life, a rigid schedule of chipping every other week throughout the year.

VIABILITY OF EGGS OF THE SWINE THORN-HEADED WORM (MACRACANTHORHYNCHUS HIRUDINACEUS) [1]

By Kenneth C. Kates

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INTRODUCTION

Although the thorn-headed worm (*Macracanthorhynchus hirudinaceus*), which parasitizes swine, is of considerable economic importance according to Schwartz,[2] there is little available information concerning the effect of environmental factors on the eggs. Therefore, an investigation was undertaken to ascertain the effects of (1) high temperatures both instantaneously applied and sustained for 10 minutes, (2) freezing in water and in a dried condition, (3) continuous drying, (4) alternate wetting and drying, and (5) ultraviolet irradiation. The investigation was carried out from July 1938 to November 1939 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

MATERIAL AND METHODS

Eggs [3] used in the tests herein described were obtained from gravid female worms collected post mortem from infected swine and dissected within 24 hours after their removal from the host animal. The eggs were first concentrated in cone-shaped sedimentation glasses and then washed at least 10 times in succession by filling the cones with tap water, allowing the eggs to sediment for 20 to 30 minutes, and then discarding the supernatant fluid, which contained some incompletely developed eggs and pieces of worm tissue. After the washing process was completed, the eggs were stored in tap water at temperatures of 2° to 5° C. until used.

The criterion for ascertaining the viability of the eggs was the capacity of the contained larvae to infect final instar grubs of the green June beetle (*Cotinis nitida* (L.)). Under experimental conditions the larval stages of this beetle are capable of serving as intermediate hosts of thorn-headed worms. The grubs were collected from soil to which hogs had not had access for 8 to 10 years, a procedure which reduced to a minimum the chances of extraneous infection of the grubs with this parasite. No thorn-headed worm larvae were ever found in control grubs selected at random from each of the various lots of grubs used.

The procedure employed in testing the viability of the eggs was as follows: Definite quantities (by volume) of eggs were thoroughly mixed with 30 cc. of slightly moist soil that had first been sifted through a 30-mesh-to-the-inch screen and then autoclaved for 30

minutes. The mixtures of soil and eggs were placed in 50-cc. metal screw-top bottles that had been sterilized in an autoclave. One grub was added to each culture and kept for 10 days at a temperature of 21° to 26° C. At the end of the 10-day period each grub was decapitated, the intestine dissected out, opened lengthwise, and then washed gently in water to remove soil particles. The intestinal tissue was then pressed between glass slides and examined microscopically for the presence of young thorn-headed worm larvae, or acanthors.[5]

In tests of the effect on eggs of high temperatures instantaneously applied, 0.5-cc. quantities of eggs were subjected to temperatures ranging from 45° to 100° C. at intervals of 5°, as follows: The eggs were pipetted into beakers each containing 100 cc. of water that had been heated to the desired temperature by means of a water bath. Immediately thereafter, the beakers were removed from the water bath and 250 cc. of ice water added to reduce rapidly the temperature of the water surrounding the eggs to approximately 30° or lower. The eggs were then concentrated by sedimentation, mixed with soil, and their viability determined by grub-feeding tests.

Another series of tests involved eggs exposed for 10 minutes to each of a series of temperatures ranging from 36° to 90° C. at intervals of 5°. Eggs in 0.5-cc. quantities were pipetted into bottles containing 10 cc. of water at the desired temperature. The bottles were then tightly stoppered and immersed in a water bath maintained at the same temperature. At the expiration of 10 minutes the bottles were removed from the bath, the stoppers drawn, the supernatant fluid quickly decanted, and the bottles filled with ice water. The viability of the eggs was then ascertained by grub-feeding tests. For both series of tests control cultures were made from 0.5-cc. quantities of eggs that had been subjected to a temperature of 26° C.

In tests involving continued freezing, 0.5-cc. quantities of eggs in a series of tightly corked sputum jars of water were frozen at temperatures of −10° to −16° C. in the freezing compartment of a mechanical refrigerator. At the same time, similar quantities of eggs that had been spread in thin layers on glass plates and then dried 10 minutes in a current of air were refrigerated to test the effect on egg viability of combined desiccation and freezing. Control preparations of eggs in water were maintained at temperatures of 21° to 26°. At intervals, as shown in table 3, preparations of eggs in water and of those in the dried condition were removed from the refrigerator, allowed to thaw at room temperature, and mixed with soil for grub-feeding tests.

In tests on the effect of continuous drying, 1 cc. of egg concentrate was spread evenly in a thin layer on the surface of each of a series of 4-inch-square glass plates and dried for approximately 10 minutes in a current of air at room temperature (26° C.). Some of the preparations were kept at temperatures of 5° to 9°, others at 21° to 26°, and still others at 37° to 39° C. At intervals, as shown in table 4, eggs from one of the preparations from each temperature group were scraped from the glass plate, mixed with moist sterile soil, and the

[5] Van Cleave[4] first used the term "acanthor" to designate both the thorn-headed worm larvae contained in eggs and the first-stage larvae in the intermediate host. As a matter of convenience the term is used throughout this paper.

number of viable eggs present in each sample estimated by grub-feeding tests.

To test the effect of alternate wetting and drying, 0.5-cc. quantities of eggs were scattered over the surface of a layer of autoclaved soil in each of a series of uncovered Petri dishes. Some of the preparations were maintained at temperatures of 2° to 5° C. and others at 37° to 39°. The preparations were kept under observation for 551 days, and during this time those at the lower temperatures were moistened five times and those at the higher temperatures nine times at irregular intervals of 30 to 150 days. The viability of the eggs was then tested by grub feeding.

In tests on the effect of ultraviolet irradiation, eggs dried on glass plates and eggs in Syracuse watch glasses in 10 cc. of water were subjected to irradiation from a quartz mercury-vapor arc lamp. Some of the dried preparations were one egg in thickness; others were several eggs thick. The cultures were irradiated at a distance of 18 inches from the tube. During irradiation the air temperature at the level of the cultures was maintained at or below 36° C. by means of a current of air. Control cultures consisting of eggs in a dried condition and in shallow water were kept at temperatures of 37° to 39° during the longest period that the test eggs were subjected to irradiation, and were not exposed to ultraviolet irradiation at any time.

EXPERIMENTAL RESULTS

EFFECT OF HIGH TEMPERATURES

In a preliminary series of 13 tests, in which the eggs were subjected to temperatures ranging from 45° to 100° C., a few apparently survived instantaneous exposure to temperatures as high as 100°, as judged by results of grub-feeding tests (table 1, tests 1 to 13). These results were not borne out in a subsequent series of 7 tests (table 1, tests 14 to 19), in which all equipment was autoclaved before being used. In

TABLE 1. *Effect on thorn-headed worm eggs of high temperatures instantaneously applied*

SERIES 1.—ALL EQUIPMENT NOT AUTOCLAVED BEFORE USE

Test No.	Temperature of water	Grubs examined	Average number of acanthors recovered per grub	Test No	Temperature of water	Grubs examined	Average number of acanthors recovered per grub
	°C.	Number			°C.	Number	
1	45	3	1,609	8	80	3	53
2	50	3	1,395	9	85	2	2
3	55	3	1,248	10	90	3	5
4	60	4	474	11	95	3	3
5	65	3	1,211	12	100	3	6
6	70	3	89	13	100	3	0
7	75	3	174				

SERIES 2.—ALL EQUIPMENT AUTOCLAVED BEFORE USE

14	50	3	1,169	18	70	6	0
15	55	4	320	19	75	3	0
16	60	4	308	20 [1]	26	1	1,544
17	65	4	3				

[1] Control.

the latter series of tests, no eggs survived instantaneous exposure to 70° or higher. It is concluded, therefore, that the apparent survival of some eggs at temperatures higher than 70° in the first series may have been due to contamination of the cultures. This may have occurred as a result of small numbers of viable eggs adhering to the dishes, pipettes, spatulas, or other equipment used in culturing the eggs after their exposure to heat.

In these tests it was observed that the majority of the eggs were unaffected, apparently, by a temperature of 45° C. This is shown by the fact that grubs fed eggs that had been subjected to this temperature harbored an average of 1,609 acanthors in the first series of tests and 1,169 in the second series, as compared with 1,544 in the control grub. In tests at higher temperatures, however, the numbers of eggs that survived decreased gradually as the temperature was raised. In the second series of tests only 3 acanthors, on the average, were recovered from the test grubs fed eggs exposed to 65° C. In this series, as already stated, no eggs survived a temperature of 70°.

In a carefully controlled series of 12 tests (table 2), in which eggs were exposed 10 minutes to temperatures ranging from 36° to 90° C., none survived at 60° or higher, except as indicated in the footnote of table 2.

TABLE 2.—*Effect on thorn-headed worm eggs of high temperatures sustained 10 minutes*

Test No.	Temperature of water	Grubs examined	Average number of acanthors recovered per grub	Test No.	Temperature of water	Grubs examined	Average number of acanthors recovered per grub
	°C	Number			°C	Number	
1	36	1	1,164	8	70	3	0
2	40	3	1,116	9	75	3	0
3	45	3	597	10	80	3	[1] 0 3
4	50	3	582	11	85	3	0
5	55	3	1,131	12	90	3	0
6	60	3	0	13 [2]	26	1	720
7	65	3	0				

[1] 1 acanthor was found in 1 grub in this test and was definitely abnormal.
[2] Controls.

EFFECT OF CONTINUOUS FREEZING IN WATER AND IN A DRIED CONDITION

As shown in table 3, continuous exposure to temperatures varying from −10° to −16° C. for periods as long as 140 days failed to destroy the viability of the majority of eggs, regardless of whether they were frozen in water or in a dried condition. With the exception of tests 7 and 8 and 11 and 12, freezing in water appeared to be somewhat more effective in reducing egg viability than freezing in a dried condition. In tests 9 to 12, the number of acanthors recovered from grubs fed eggs at intervals during this period was comparable to the number recovered from corresponding control grubs. The smaller number of acanthors recovered from tests 1 to 8 was probably due to a difference in activity of the grubs used.

TABLE 3.— *Effect on thorn-headed worm eggs of freezing in water and in a dried condition* [1]

Test No.	Condition of eggs at time of freezing	Period of exposure to freezing (−10° to −16° C.)	Grubs examined	Average number of acanthors recovered per grub	Test No.	Condition of eggs at time of freezing	Period of exposure to freezing (−10° to −16° C.)	Grubs examined	Average number of acanthors recovered per grub
		Days	*Number*				*Days*	*Number*	
1	In water	4	2	173	8	Dried	42	1	140
2	Dried	4	2	284	9	In water	81	1	796
3	In water	11	2	310	10	Dried	81	1	1,496
4	Dried	11	1	562	11	In water	140	1	902
5	In water	24	2	322	12	Dried	140	1	721
6	Dried	24	2	459	13 [2]		0	5	810
7	In water	42	1	164					

[1] The dried eggs, before being frozen, had been spread in thin layers on glass plates and exposed to air for 10 minutes.
[2] Controls in water at 21° to 26° C.

EFFECT OF CONTINUOUS DRYING

The data presented in table 4 show that continuous drying at temperatures of 21° to 26° C. for 265 days and at 5° to 9° and 37° to 39° for 50 days failed to destroy the viability of the eggs. Data already presented in table 3 show that the majority of eggs exposed in a dried condition to temperatures ranging from −10° to −16° survived for as long as 140 days. These findings indicate that continuous drying, which is effective in destroying the viability of eggs and larvae of some parasites, is comparatively ineffective in destroying the viability of eggs of the swine thorn-headed worm.

In tests 3 to 6, the numbers of acanthors recovered from the grubs were noticeably smaller than those in the remainder of the tests. This difference was due to the fact that the temperatures at which the grubs were kept were lowered to 7° to 15° C., because of an unforseen reduction in laboratory temperature. Under these conditions the grubs became partially inactive, did not feed readily, and, therefore, may not have ingested so many eggs as those kept at higher temperatures.

TABLE 4.— *Effect of continuous drying on thorn-headed worm eggs*

Test No	Period of exposure to drying	Exposure temperature	Grubs examined	Average number of acanthors recovered per grub	Test No.	Period of exposure to drying	Exposure temperature	Grubs examined	Average number of acanthors recovered per grub
	Days	*°C*	*Number*			*Days*	*°C*	*Number*	
1	16	5–9	1	1,778	8	35	21–26	1	2,789
2	16	21–26	2	1,467	9	35	37–39	1	1,788
3	16	37 39	2	817	10	50	5–9	2	1,354
4	25	5–9	1	550	11	50	21–26	2	1,111
5	25	21–26	1	394	12	50	37 39	2	1,507
6	25	37–39	1	346	13	265	21–26	1	1,027
7	35	5–9	1	1,004	14 [1]	0		5	1,453

[1] Controls.

EFFECT OF ALTERNATE WETTING AND DRYING

The data presented in table 5 show that the viability of practically all eggs on soil subjected to alternate wetting and drying at temperatures of 37° to 39° C. were destroyed in 368 days and that all eggs were destroyed in 551 days. At temperatures of 2° to 5°, however, comparatively large numbers of acanthors were recovered at the end of these periods. Manter [6] and others have observed that when eggs are dried and then placed in water some of the shells break, thereby allowing the acanthors to escape. This observation has also been made by the writer and may explain the loss of egg viability in tests in which the samples were subjected to repeated wetting and drying at 37° to 39° (tests 2, 4, 6, and 8 in table 5). The fact that at 2° to 5° the soil of the samples did not become so thoroughly dry as that at 37° to 39° accounts for the greater survival of eggs under these conditions.

TABLE 5.—*Effect of alternate wetting and drying on thorn-headed worm eggs*

Test No	Period of exposure	Exposure temperature	Grubs examined	Average number of acanthors recovered per grub	Test No.	Period of exposure	Exposure temperature	Grubs examined	Average number of acanthors recovered per grub
	Days	*°C*	*Number*			*Days*	*°C.*	*Number*	
1	102	2-5	1	2, 852	6	368	37–39	1	1
2	102	37–39	1	276	7	551	2-5	2	1, 194
3	132	2–5	1	727	8	551	37–39	2	0
4	132	37–39	1	352	9 [1]	551	2-5	1	270
5	368	2-5	1	272					

[1] Control continuously dry.

EFFECT OF ULTRAVIOLET IRRADIATION

Table 6 summarizes the data on the effect of ultraviolet irradiation on eggs of the thorn-headed worm. Tests 1 to 11 show the effect of the irradiation on dry eggs spread in several layers on glass plates. In all cases, as compared with the controls, there was some reduction in the number of acanthors recovered, although exposures up to 40 minutes failed to destroy viability in all the eggs. When eggs were spread on glass plates in a single layer by using smaller quantities of egg concentrate and then exposed to irradiation (tests 12 to 32), only an insignificant number survived a 10-minute exposure. This finding indicates that direct exposure to strong ultraviolet irradiation will render the eggs of this parasite nonviable very quickly. Eggs placed in shallow water and then irradiated (tests 33 to 51) showed a tendency to decrease gradually in viability as the period of irradiation was lengthened. Some eggs, however, survived irradiation for periods of 120 minutes (test 50).

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[6] MANTER. H. W. NOTES ON THE EGGS AND LARVAE OF THE THORNY-HEADED WORM OF HOGS. Amer. Micros. Soc. Trans. 47: 342–347. illus. 1928.

TABLE 6.—*Effect of ultraviolet irradiation*

EGGS DRIED IN SEVERAL LAYERS ON GLASS PLATES

Test No.	Quantity of egg concentrate	Period of exposure	Grubs examined	Average number of acanthors recovered per grub
	Cc	Minutes	Number	
1 ...		30	2	1,077
2	1	40	2	267
3		0	2	1,770
4		5	2	342
5		10	2	144
6		15	2	122
7	0 5	20	2	41
8		25	2	129
9		30	2	89
10		35	2	118
11 [1]		0	2	419

EGGS DRIED IN SINGLE LAYER ON GLASS PLATES

Test No.	Quantity of egg concentrate	Period of exposure	Grubs examined	Average number of acanthors recovered per grub
12	0 2	10	2	1
13		20	2	0
14-23		2 30-120	12	0
24 [1]		0	2	1,097
25		10	2	3
26		20	2	1
27		30	2	0
28	0 01	40	2	2
29		50	2	0
30		60	4	0
31		70	4	0
32 [1]		0	2	262

EGGS IN WATER

Test No.	Quantity of egg concentrate	Period of exposure	Grubs examined	Average number of acanthors recovered per grub
33		10	2	237
34		20	2	15
35	0 05	30	1	6
36		40	2	0
37		50	2	5
38 [1]		0	1	383
39		10	1	882
40		20	1	1,106
41		30	1	528
42		40	1	831
43		50	1	516
44		60	1	79
45	0 2	70	1	1,342
46		80	2	101
47		90	2	2
48		100	1	88
49		110	1	21
50		120	1	208
51 [1]		0	2	1,042

[1] Controls.
[2] 10-minute intervals.

SUMMARY AND CONCLUSIONS

Tests under laboratory conditions were carried out on the effects of high and low temperatures, drying, alternate wetting and drying, and ultraviolet irradiation on the viability of eggs of the swine thorn-headed worm (*Macracanthorhynchus hirudinaceus*). The criterion used to test the viability of the eggs was the ability of the contained larvae, or acanthors, to infect grubs of the green June beetle (*Cotinis nitida* (L.)). The work was carried on in 1938 and 1939 at the

United States Department of Agriculture, Beltsville Research Center, Beltsville, Md.

Eggs in water were destroyed by instantaneous exposure to a temperature of 70° C. and by 10 minutes' exposure to a temperature of 60°. In one series of preliminary tests, not so well controlled, a small number of eggs survived higher temperatures.

The majority of eggs in water and in a dried condition survived continuous exposure to temperatures ranging from −10° to −16° C. for as long as 140 days, when the test was terminated.

No appreciable reduction in number of viable eggs was observed in dry preparations exposed for 50 days at temperatures of 5° to 9° C. and 37° to 39°, and for 265 days at temperatures of 21° to 26°.

Alternate wetting and drying at temperatures of 37° to 39° C. destroyed the viability of eggs on soil in 368 days. On the other hand, eggs subjected to alternate wetting and drying at temperatures of 2° to 5° were still viable after 551 days.

Ultraviolet irradiation applied at a distance of 18 inches destroyed nearly all the dry eggs in a single layer in 10 minutes. Some eggs in films several eggs thick survived for 40 minutes. Some eggs in shallow water survived irradiation for as long as 120 minutes.

Data presented in this paper on the resistance, to experimental conditions, of eggs of thorn-headed worms furnish, in part, an explanation for the fact that eggs of this parasite have remained viable on soil for as long as 3½ years under conditions at Beltsville, Md., as reported in previous work.[7]

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[7] SPINDLER, L A., and KATES, K. C. SURVIVAL ON SOIL OF EGGS OF THE SWINE THORN-HEADED WORM, MACRACANTHORHYNCHUS HIRUDINACEUS. (Abstract) Jour. Parasitol. 26 (6) Sup. · 19   1940.

INHERITANCE OF SMOOTH AND PITTED BOLLS IN PIMA COTTON [1]

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INTRODUCTION

In cotton relatively few characters segregate in simple Mendelian fashion. Kearney [2] lists only 14 allelic pairs of characters. Additions have been made since his list was compiled, but the number is still small. Conspicuous allelic characters, if not deleterious, are of interest to cotton breeders for the reason that they can be transferred from one variety to another and utilized as a mark of identification. The discovery of a character of this nature, a smooth-boll variation in Pima cotton, is reported in this paper.

OBSERVATIONS AND EXPERIMENTAL WORK

The boll surface in Pima, an American-Egyptian variety of *Gossypium barbadense* L., is dotted with craterlike pits (fig. 1, *A*). A large
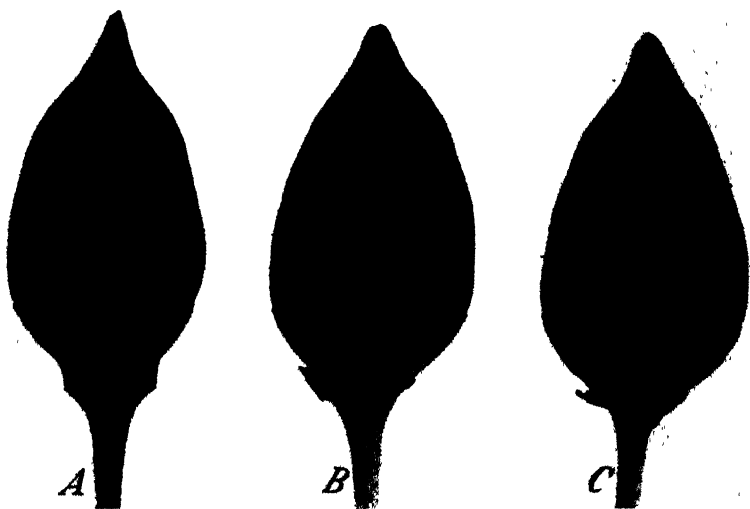
FIGURE 1.—Pitted, intermediate, and smooth cotton bolls: *A*, Normal Pima, pitted; *B*, Pima × P Hope F₁, showing intermediate pitting; *C*, P Hope, smooth. × about 1¼ (bolls approximately 40 mm. long).

oil or resin gland, which bleeds freely when bruised, is embedded at the base of each pit. In the variant smooth-boll form the bolls appear to be smooth (fig. 1, *C*), the pits being very obscure. Also, the glands are smaller than in typical Pima and do not bleed so freely when the surface of the boll is bruised.

The parent of the smooth-boll strain was found in 1932, by Claude Hope,[3] in a field of Pima cotton that was grown for increase of pure planting seed at the United States Field Station, Sacaton, Ariz. A few hybrids between *Gossypium barbadense* and *G. hirsutum* L. appear at Sacaton every year in the fields that are used for increasing seed, so there is a possibility that the parent of the smooth-boll strain was a dilute hybrid of this origin. However, for convenience the strain is treated herein as *G. barbadense*, as the origin of the parent plant will always remain conjectural. The plant was much like Pima, except for the conspicuously different character of the boll surface.

Only open-pollinated seeds were obtained in 1932, there having been no opportunity to protect the flowers against cross-pollination. Nevertheless, in the following year all of the progeny produced smooth bolls. The strain, which was designated "P Hope," has bred true for eight generations.

In 1933 the smooth-boll strain was crossed with normal Pima. The surface of the bolls proved to be intermediate in degree of pitting in all individuals of the first generation. In subsequent years, second-generation and third-generation populations were grown, as well as several backcrosses. The classifications of the several populations are given in table 1.

Segregation in F_2, and in F_3 of an intermediate F_2 individual, approximated a 1 : 2 : 1 ratio, and segregation in backcrosses of F_1 individuals with individuals of the pitted and smooth parental families, respectively, approximated a 1 : 1 ratio. The departure of the observed distribution from the distribution calculated on the assumption of a single-factor difference is in no instance significant. It seems clear, therefore, that smooth-boll inheritance is monohybrid in this intraspecies cross.

TABLE 1. — *Classification of the F_1, F_2, F_3, and backcross populations in a cotton cross involving smooth and pitted boll surface* [1]

Combination	Year	Classification of parent	Number of plants	Observed distribution			x^2	P
				Smooth	Intermediate	Pitted		
First crosses:								
Smooth × pitted F_1	1934	....	29	0	29	0		
Smooth × pitted F_2	1935	Intermediate	50	11	28	11		
Do	1938	do	68	13	36	19		
Do	1940	do	53	12	32	9		
Combined F_2 populations			171	36	96	39	2.68	0.3
Smooth × pitted F_2	1936	Smooth	9	9	0	0		
Do	1939	do	41	41	0	0		
Do	1936	Pitted	10	0	0	10		
Do	1936	Intermediate	26	6	14	6	.15	.9
Backcrosses								
Intermediate × pitted F_1	1936		29	0	12	17		
Do	1937		34	0	14	20		
Combined populations			63	0	26	37	1.92	.2
Intermediate × smooth F_1	1936		28	10	18	0		
Do	1937		34	21	13	0		
Combined populations			62	31	31	0		

[1] The values for x^2 of the departures from a 1 : 2 : 1 and a 1 : 1 ratio, respectively, are computed on the assumption of a single-factor difference. For formula see FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., 269 pp., illus. 1928.

[3] Then a member of the staff of the Division of Cotton, Rubber, and Other Tropical Plants (now the Division of Cotton and Other Fiber Crops and Diseases), Bureau of Plant Industry.

DISCUSSION

Although the presence or absence of pits on the bolls is of taxonomic importance in the genus *Gossypium*, the inheritance had not been analyzed successfully previous to the present study, according to Kearney [4] and Harland [5] (*pp. 117–118*). Harland (*p. 58*) suggests that Mendelian segregation may be obscured in interspecific cotton crosses by the "large number of different genetical backgrounds upon which the given pair of alleles would manifest themselves." This hypothesis offers an explanation for the difficulty encountered by investigators who have worked with interspecific hybrid populations. The present success with the pitted-boll character may be accounted for on the assumption of similar genetic backgrounds in P Hope and ordinary Pima. Even though P Hope may have originated by natural hybridization between Pima (*G. barbadense*) and upland cotton (*G. hirsutum*), a Pimalike genetic background could have been acquired by repeated natural backcrossing on Pima prior to the discovery of the parent P Hope plant.

The symbols B^p and B^s, respectively, are proposed for the allelic boll surface characters pitted and smooth.

[4] KEARNEY, THOMAS H   SEGREGATION AND CORRELATION OF CHARACTERS IN AN UPLAND-EGYPTIAN COTTON HYBRID. U S Dept Agr Dept Bul 1164, 57 pp , illus   1923
[5] HARLAND, SYDNEY CROSS   THE GENETICS OF COTTON   193 pp , illus   London   1939

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INHERITANCE OF CLUSTER HABIT AND ITS LINKAGE RELATION WITH ANTHOCYANIN PIGMENTATION IN UPLAND COTTON [1]

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INTRODUCTION

Future progress in the improvement of the cotton plant by breeding is dependent to some extent upon a more complete genetic analysis of the various wild and cultivated species. The accumulation of knowledge regarding the mode of inheritance of characters, the identification of these characters with the chromosomes, and the mapping of the chromosomes will increase the certainty and rapidity of cotton improvement.

Genetic studies in New World cottons have resulted in definite information regarding the mode of inheritance of forty-odd factors that affect 14 characteristics. Among these are the genes that affect the anthocyanin pigmentation and cluster habit.

The presence of anthocyanin results in a red or wine-red coloration in cotton plants. Some degree and type of anthocyanic pigmentation occurs in practically every species of the genus *Gossypium*. In *G. hirsutum*, according to Ware's (18)[2] description, all exposed parts of plants homozygous for the characteristic possess a deep red color. In the heterozygous condition the color is a dilute red, and the anthocyanin pigment is somewhat restricted to the plant stems, the petioles and veins of the leaves, and the portion of the corolla exposed to sunlight; while the red coloration of the leaf lamina is very dilute.

McLendon (12), Thadani (17), Ware (18), Carver (1), and Harland (4) have shown that anthocyanin pigmentation is controlled by a single genetic-factor pair. Ware (19, 20) found that the factors for red plant and "okra leaf," for red plant and green lint, and for red plant and brown lint segregate independently. Killough and Horlacher (11) reported an independent assortment for the genes affecting the red-plant and virescent-yellow characteristics.

Certain strains of upland cotton are characterized by an excessive shortening of the fruiting branches, and the bolls are borne in clusters. Thadani (17) found that an intra-upland cross between cluster and noncluster types resulted in a normal F_1 and a segregation of 3 normal to 1 cluster in the F_2 generation, indicating that a single factor pair was involved. Texas workers (16, p. 54) found a cluster type in the Durango variety and reported that when crossed with a noncluster type it behaved as a recessive. Harland (6) found that cluster in some crosses behaved in a complicated manner, showing all grades of blending between cluster and normal in the F_2 segregation, and con-

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cluded that the cluster habit is determined by one basic gene and that modifiers may determine minor differences in the degree to which cluster is expressed.

In summarizing the heritable relationships of characteristics in New World cottons, Harland (6) indicated that the genes affecting virescent-yellow and cluster habit are inherited independently.

Only two definite cases of linkage in the New World cottons have been reported. However, the heritable relationships of only 56 pairs of genes have been recorded; and since there are 26 (haploid) chromosomes, it is not surprising that more cases of linkage have not been established.

Thadani (17) studied the heritable relationships of anthocyanin pigmentation and cluster habit in the F_2 generation of a red plant, cluster habit $\times$ green plant, noncluster habit cross, and found linkage. The calculation of cross-over values from Thadani's data is not easily accomplished, since the monohybrid ratios were rather disturbed and since no green-cluster recombination phenotypes were obtained in the segregation. Harland (5), who studied the linkage relationship in the backcross segregation, found that the anthocyanin and cluster genes belong to the same linkage group and obtained a cross-over value of 13.9 percent.

Harland (5) has reported another case of linkage in New World cottons. He found in a small backcross population that crinkled and green lint were linked with 5 percent crossing over. Three clear cases of linkage in Asiatic cottons have been reported. Hutchinson (7) found that the genes for leaf shape and brown lint were linked and that 29.9 percent cross-overs were obtained. Yu (21) has reported that yellow seedling and anthocyanin pigmentation are linked and that the cross-over value is about 9 percent. Yu (22) also found that curly leaf and virescent bud in Asiatic cotton are linked, with a cross-over value not far from 16.6 percent.

In the cotton genetic studies now being conducted by the Bureau of Plant Industry, in cooperation with the Mississippi Agricultural Experiment Station, the heritable relations of a large number of characteristics are being determined. Since the mode of inheritance of the cluster habit of strains isolated from certain varieties has not been reported, and since, in order to be of predictive value, recombination fractions should be based upon the results of several repetitions, the studies regarding the mode of inheritance of the cluster-habit character and the chromosomal relationships of cluster habit and red plant have been repeated. The results presented here are not considered conclusive, nor are they presented to refute the work of earlier investigators. For the most part, the findings of these geneticists have been verified, and when divergent conclusions are expressed, they are offered mainly for widening the scope of investigation and as a stimulation to further studies regarding these genetic phenomena.

MATERIALS AND METHODS

Two strains of *Gossypium hirsutum* L. were used in these studies. The Winesap, anthocyanin-pigmented type (fig. 1, *A*), was obtained from the Arkansas Agricultural Experiment Station and had been inbred for eight generations. The Bawaka, cluster-habit type (fig. 1, *B*), was obtained through the Division of Plant Exploration and Intro-

duction of the Bureau of Plant Industry from the Turkestan Plant Breeding Station, Tashkent, U. S. S. R., and, no doubt, is a derivative of an introduced American upland variety. The phenotypically red, noncluster strain has been designated genotypically as $R_1^{Ro}R_1^{Ko}ClCl$, and the phenotypically green, cluster strain has been designated genotypically as $r_1^{ro}r_1^{ro}clcl$, according to the gene symbols proposed by Hutchinson and Silow (8). The superscript indicates that the effect of the R_1 gene does not extend to the petal spot, as does the effect of certain duplicate anthocyanin genes.

The methods of selecting parent plants and of self- and cross-pollination were the same as described by Ware (18). Crosses were made in 1936 and 1937 between the Winesap and Bawaka lines. The Winesap was used as the female parent in all crosses, since emasculation is more difficult and shedding is greater in the Bawaka strain. Progenies were grown in 25-foot rows, each containing 20 to 25 hills. In those progenies that segregated for anthocyanin pigmentation the proportion of red, intermediate red, and green plants was verified, and the progenies were then thinned to a 1 : 2 : 1 ratio in the F_2 and F_3 populations and to a 1 : 1 ratio in the backcross population. This method of planting and thinning was necessary, since singly spaced seed have difficulty in germinating because of the compact surface of the soil and thickly spaced plants do not develop sufficiently for accurate classification of the cluster habit.

Upon maturity of the plants the frequencies of each of the following phenotypes in the F_2 and F_3 generations were determined: Red noncluster, intermediate-red noncluster, green noncluster, red cluster, intermediate-red cluster, and green cluster. The backcross segregates were classified into four phenotypic classes: Intermediate-red noncluster, intermediate-red cluster, green noncluster, and green cluster. To facilitate linkage calculations, the intermediate-red noncluster segregates were placed in the red noncluster phenotypic class, and the intermediate-red cluster segregates were placed in the red cluster phenotypic class. The F_2 segregates were divided into four families and the F_3 segregates into three families on the basis of different individual plants that were used in the parental combinations. However, there is no obvious reason for adopting this grouping, since all male and all female parents were from the same respective inbred line. Both F_2 and F_3 progenies of double heterozygous plants were used in determining the linkage values. One family of backcross segregates was propagated and analyzed.

INHERITANCE OF CLUSTER HABIT

F_1 POPULATIONS

The F_1 generations of the noncluster $\times$ cluster cross, grown in 1937 and 1938, were composed of 12 and 16 plants, respectively. These plants (fig. 1, *C*) were classified as noncluster, since there was no obvious shortening of the sympodial branches. However, there appeared to be a rather consistent difference between these F_1 plants and the noncluster parental line. In the axis of the branch and the regular flower pedicel of the F_1 plants, an axillary fruiting bud formed. These "squares" were shed before they developed into flowers; and when the plant was mature, it was morphologically similar to the noncluster parent.

FIGURE 1.— Parents and F_1 and F_2 of hybrid upland cotton: *A*, Noncluster parent $R_1{}^{RO}$; *B*, cluster parent $r_1{}^{ro}$; *C*, F_1 of cross between *A* and *B* is noncluster; F_2 progeny segregates into three noncluster (*D*) and one cluster (*E*).

F₁ POPULATIONS

The F_1 plants were self-pollinated; in 1938 and 1939, 672 F_2 plants were grown to maturity. These progenies segregated into two distinct types, noncluster and cluster (fig. 1, D and E), respectively. In table 1 the phenotypic-class frequencies of the F_2 generation of the four families, the test for goodness of fit with the expected 3 : 1 ratio, and the test for agreement between families are given. The component of χ^2 attributed to the heterogeneity between families was obtained by subtracting the χ^2 for deviation from the sum of the χ^2's attributed to the four families, according to the procedure suggested by Mather (*13*). The observed and calculated frequencies agree quite closely; the probability value, according to Fisher's (*2*) table of χ^2 being 0.61. The segregations within the families agree with one another, the probability value being 0.57. These two agreements indicate that the cluster and noncluster characteristics differ by one genetic-factor pair.

TABLE 1.—*Phenotypic-class frequencies of the F_2 generation of the noncluster $\times$ cluster cross; the test for goodness of fit with the expected ratio; and the test for agreement between families*

Family	*Cl*	*cl*	Total	χ^2	Degrees of freedom	Probability
1	16	7	23	.3623	1	
2	62	27	89	1 3520	1	
3	100	38	138	.4734	1	
4	320	102	422	.1548	1	
Total	498	174	672	2 3425	4	
Calculated (3 1)	504	168	672			
Deviation				2857	1	.61
Heterogeneity				2 0568	3	.57

BACKCROSS POPULATION

In table 2 the frequencies of the Cl and cl phenotypes of the backcross $(r_1{}^{ro}cl/R_1{}^{RO}Cl \times r_1{}^{ro}cl/r_1{}^{ro}cl)$, and the test for goodness of fit with the expected 1 : 1 ratio are given. The observed and calculated frequencies agree satisfactorily, the probability value being 0.50.

TABLE 2.— *Phenotypic-class frequencies of the backcross $(r_1{}^{ro}cl/R_1{}^{RO}Cl \times r_1{}^{ro}cl/r_1{}^{ro}cl)$ and the test for goodness of fit with the expected ratio*

Phenotypic class	Observed	Calculated (1.1)	χ^2	Degrees of freedom	Probability
Cl	66	70			
cl	74	70			
Total	140	140	.457	1	.50

DETECTION OF LINKAGE

F₂ AND F₃ POPULATIONS

The frequencies of the four phenotypic classes of the F_2 generation of families 1, 2, 3, and 4, and of the F_3 segregation of the F_2 double heterozygotes of families 1, 2, and 3, were used in the detection of linkage.[3] The χ^2 for the segregation of these double heterozygous

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[3] While the frequencies of some F_3 lines were somewhat smaller than was desirable, the lines were tested to determine whether they came from coupling or repulsion recombination, as suggested by Immer (*10*). None of the F_3 lines used in these studies conformed satisfactorily to the repulsion-recombination hypothesis.

$(R_1{}^{RO}r_1{}^{ro}Clcl)$ plants includes three components: (1) Deviation of the $R_1{}^{RO}$, $r_1{}^{ro}$ segregation from its 3 : 1 expectation, (2) deviation of the Cl, cl segregation from the single-factor expectation, and (3) the deviation of the joint segregation from its expectation of independent inheritance. The following formulas, which are discussed by Mather (*13*), were used in calculating the three χ^2 values:

$$\chi^2 R_1{}^{RO} = (R_1{}^{RO}Cl + R_1{}^{RO}cl - 3r_1{}^{ro}Cl - 3r_1{}^{ro}cl)^2/3n$$

$$\chi^2 Cl = (R_1{}^{RO}Cl - 3R_1{}^{RO}cl + r_1{}^{ro}Cl - 3r_1{}^{ro}cl)^2/3n$$

$$\chi^2 \text{ linkage} = (R_1{}^{RO}Cl - 3R_1{}^{RO}cl - 3r_1{}^{ro}Cl + 9r_1{}^{ro}cl)^2/9n$$

The first two components, corresponding to single-factor ratios, are based upon the formula for calculating the χ^2 values for the 3 : 1 segregation; the third component follows from orthogonality.

The goodness of fit tests were made with two groupings of the phenotypic frequencies, the family grouping and the generation grouping. In each analysis the χ^2 was divided into two parts: (1) That concerned with the deviation of all groups taken together from the expected ratio, and (2) that concerned with the lack of agreement between the groups.

The phenotypic-class frequencies of the combined F_2 and F_3 progenies, the tests for goodness of fit for the single-factor and joint segregations for each family and for the combined populations, and the tests for agreement between families are given in table 3. The thinned population of red and green phenotypes corresponds closely to the expected 3 : 1 ratio, the probability value being 0.44. The segregation into Cl and cl phenotypic classes conformed satisfactorily to the single-factor hypothesis, the probability value being 0.67. In each case the heterogeneity between families was not significant, the probability values for $R_1{}^{RO}$ and Cl being 0.74 and 0.61, respectively. The χ^2 for linkage, 324.3630, corresponds to a very small probability value, and it is thus quite obvious that the single-factor ratios account for very little of the total χ^2 but that there is a large component corresponding to linkage. The heterogeneity χ^2 for linkage was not significant, the probability value being 0.42. Thus the four families agree in showing linkage of the $R_1{}^{RO}$ and Cl factors.

TABLE 3.—*Phenotypic-class frequencies of combined F_2 and F_3 progenies of the cross red noncluster × green cluster; the tests for goodness of fit for the single-factor and joint segregations for each family and for the combined populations; and the tests for agreement between families*

Family	Frequencies				Degrees of freedom	$R_1{}^{RO}r_1{}^{ro}$ segregation		$Clcl$ segregation		Joint segregation	
	$R_1{}^{RO}Cl$	$R_1{}^{RO}cl$	$r_1{}^{ro}Cl$	$r_1{}^{ro}cl$		χ^2	Probability	χ^2	Probability	χ^2	Probability
1	80	9	12	19	1	.04444		.1778		32.7259	
2	175	30	24	48	1	.1456		1.4741		79.4324	
3	122	20	14	29	1	.3045		.2180		47.4240	
4	282	24	38	78	1	1.3934		.1548		167.6682	
Total	659	83	88	174	4	1.8879		2.0247		327.2505	
Calculated 9 : 3 : 3 : 1	564.75	188.25	188.25	62.75							
Deviation					1	.6428	.44	.1912	.67	324.3630	.00
Heterogeneity					3	1.2451	.74	1.8355	.61	2.8875	.42

In table 4 the phenotypic-class frequencies of the combined four families of F_2 progenies and combined three families of F_3 progenies, the tests for goodness of fit for the single-factor and joint segregations for each generation and for the combined populations, and the tests for agreement between generations are given. The χ^2 and probability values for deviation for each of the single-factor segregations and for the joint segregation are the same as given in table 3. For none of the three segregations is the heterogeneity χ^2 significant, and it is shown that the two generations are homogeneous for each component of the total χ^2.

TABLE 4. *Phenotypic-class frequencies of combined four families of F_2 progenies and three families of F_3 progenies of the cross red noncluster $\times$ green cluster; the tests for goodness of fit for the single-factor and joint segregations for each generation and for the combined populations; and the tests for agreement between generations*

Generation	Frequencies				Degrees of freedom	$R_1^{RO}r_1^{ro}$ segregation		$Clcl$ segregation		Joint segregation	
	$R_1^{RO}Cl$	$R_1^{RO}cl$	$r_1^{ro}Cl$	$r_1^{ro}cl$		χ^2	Probability	χ^2	Probability	χ^2	Probability
F_2	441	53	57	121	1	.7937	.	2857	.	238.0952	.
F_3	218	30	31	53	1	.0161	.	.0000	.	87.7323	.
Total	659	83	88	174	2	.8098	.	.2857	.	325.8275	.
Calculated 9 3 3 1	564.75	188.25	188.25	62.75							
Deviation					1	.6428	44	.1912	67	324.3630	.00
Heterogeneity					1	1670	.69	.0945	76	1.4645	.23

BACKCROSS POPULATION

The detection of linkage was applied also to the backcross progeny. The number of segregates is very small, but the reduction of the data should be of some value as a supplement to the determinations made from the F_2 and F_3 progenies. The following formulas, referred to by Mather (*13*), were used in calculating the χ^2 values for the three components:

$$\chi^2 R_1^{RO} = (R_1^{RO}Cl + R_1^{RO}cl - r_1^{ro}Cl - r_1^{ro}cl)^2/n$$

$$\chi^2 Cl = (R_1^{RO}Cl - R_1^{RO}cl + r_1^{ro}Cl - r_1^{ro}cl)^2/n$$

$$\chi^2 \text{ linkage} = (R_1^{RO}Cl - R_1^{RO}cl - r_1^{ro}Cl + r_1^{ro}cl)^2/n$$

The phenotypic-class frequencies and the test of goodness of fit for the single-factor and joint segregations are given in table 5. The deviations of the R_1^{RO} and Cl segregations from expectation were not significant, the probability values being 0.18 and 0.50, respectively. However, the χ^2 for the joint segregation is highly significant, and it is shown that the phenotypic frequencies do not conform to those expected for independent inheritance.

Since in both groupings of the F_2 and F_3 phenotypic frequencies and in the backcross generation the single-factor ratios account for a very small part of the total χ^2, and the large component corresponds to the joint segregation, there is undoubtedly evidence of linkage of the R_1^{RO} and Cl factors.

TABLE 5.—*Phenotypic-class frequencies of the backcross* $(r_1{}^{ro}cl/R_1{}^{RO}Cl \times r_1{}^{ro}cl/r_1{}^{ro}cl)$ *and the test for goodness of fit for the single-factor and joint segregations*

Values observed or calculated	Frequencies				De- grees of free- dom	$R_1{}^{RO}r_1{}^{ro}$ segregation		$Clcl$ segre- gation		Joint segre- gation	
	$R_1{}^{RO}Cl$	$R_1{}^{RO}cl$	$r_1{}^{ro}Cl$	$r_1{}^{ro}cl$		χ^2	Prob- abil- ity	χ^2	Prob- abil- ity	χ^2	Prob- abil- ity
Observed ___	52	10	14	64	1	1.829	.18	457	.50	60 457	.00
Calculated 1.1:1.1	35	35	35	35		____		_ _ __		_	_____

ESTIMATION OF LINKAGE VALUES

F₂ AND F₃ POPULATIONS

As pointed out by Sturtevant and Beadle (*15*), the estimation of linkage values from data of a backcross to the double-recessive parental line is more direct and more efficient than the estimation of values from F_2 or F_3 data. However, in cotton only a few seeds are obtained from each pollination, and it is difficult to obtain a large backcross population. Since the linkage under study is not very strong and since the recessive allelomorphs are both contributed by the same parent, it is thought that the calculation of the recombination percentages from the F_2 and F_3 data is not particularly inefficient.

The linkage values, based upon the recombination frequencies from the F_2 and F_3 data, were estimated by the use of the product formula described by Fisher and Balmukand (*3*) and by Immer (*9*). Product-ratio values were determined, and the corresponding recombination fractions were obtained from tables prepared by Stevens (*14*). The percentage of recombinations, as estimated from the combined F_2 and F_3 progenies of the four families, is 18.5.

THE BACKCROSS POPULATIONS

The linkage values, based upon the recombination frequencies of the backcross, were estimated from the small population of 140 segregates. Calculations resulted in a cross-over value of 17.1 percent.

HETEROGENEITY OF THE GROUPS IN RESPECT TO THE RECOMBINATION FRACTIONS

F₂ AND F₃ POPULATIONS

By assuming homogeneity, the cross-over value 0.18499, which was estimated from the total F_2 and F_3 population, may be used in the formulation of the expected segregation of the various groups. The expected frequencies of the four phenotypic classes of the F_2 and F_3 generations were determined by the following formulas:

$$R_1{}^{RO}Cl = \frac{n}{4}(2+P)$$

$$R_1{}^{RO}cl = \frac{n}{4}(1-P)$$

$$r_1{}^{ro}Cl = \frac{n}{4}(1-P)$$

$$r_1{}^{ro}cl = \frac{n}{4}P$$

where $P = (1-p)^2$ and p is the recombination fraction.

In table 6 the goodness of fit test, based upon the agreement between the observed F_2 and F_3 phenotypic frequencies of the individual families and the expected frequencies, assuming the recombination fraction of the combined populations, is given. Following the suggestion of Mather (*13*), the two singly dominant classes of each family were added together for purposes of estimation, since they have the same expectation in terms of P. The contributions of the four families to the χ^2 are 0.0794, 1.7848, 0.3872, and 2.0031, making a total of 4.2545. There are seven degrees of freedom, since the three classes of each family contributed two degrees, one of the total of eight being lost in estimating the linkage value. The resulting probability value is 0.75, and it is concluded that the deviation of the four families from expectation is not significant.

TABLE 6.— *Goodness of fit test, based upon the agreement between the observed F_2 and F_3 phenotypic frequencies of the individual families and the expected frequencies, assuming the recombination fraction of the combined populations* $(p = 0.66424)$

Family		Frequencies		$(O-C)^2/C$
		Observed	Calculated	
1		80	79.9272	0.0001
		21	20.1456	.0362
		19	19.9272	.0431
Total		120	120.0000	.0794
2		175	184.4986	.4890
		54	46.5028	1.2087
		48	45.9986	.0871
Total		277	277.0000	1.7848
3		122	123.2211	.0121
		34	31.0578	.2787
		29	30.7211	.0964
Total		185	185.0000	.3872
4		282	281.0773	.0030
		62	70.8454	1.1044
		78	70.0773	.8957
Total		422	422.0000	2.0031
Total χ^2				4.2545

[1] Degrees of freedom = 7, probability = 0.75.

In table 6 the F_2 and F_3 segregating progenies were combined in three of the families. Such combining of generations is not justified unless it is known that there is no significant difference between the F_2 and F_3 recombination fractions. The goodness of fit test, based upon the agreement of the observed phenotypic frequencies of the F_2 and F_3 generations and the expected frequencies, assuming the recombination fraction of the combined populations, is given in table 7. The χ^2 values for the F_2 and F_3 segregates are 0.9605 and 0.6239, respectively, making a total of 1.5844. There are three degrees of freedom, and the probability value is 0.67. The observed phenotypic frequencies of the F_2 and F_3 segregations agree satisfactorily with the assumed frequencies, based upon the cross-over value of 18.5 percent.

TABLE 7.— *Goodness of fit test, based upon the agreement between the observed pheno-typic frequencies of the combined F_2 and F_3 generations and the expected frequencies, assuming the recombination fraction of the combined populations ($p = 0.66424$)*

Generation		Frequencies		$\frac{\chi^2}{(O - C)^2/C}$
		Observed	Calculated	
F_2		441	447 5923	0 0971
		110	112 8154	0703
		121	111. 5923	7931
Total		672	672 0000	. 9605
F_3		218	221. 1319	0444
		61	55. 7362	4971
		53	55. 1319	0824
Total		332	332 0000	6239
Total χ^2				[1] 1 5844

[1] Degrees of freedom = 3; probability = 0 67.

TABLE 8. — *Goodness of fit test, based upon the agreement between the observed F_2 and F_3 frequencies of the individual families and the expected frequencies, assuming the recombination fraction of the respective family*

Family	$P = (1 - p)^2$	Frequencies		$\frac{\chi^2}{(O - C)^2/C}$
		Observed	Calculated	
1	65012	80	79 5036	0 0031
		21	20 9928	0000
		19	19 5036	0130
Total		120	120 0000	0161
2	62500	175	181 7813	2530
		54	51 9375	0819
		48	43 2812	5145
Total		277	277 0000	8494
3	63581	122	121 9062	0001
		34	33 6876	0029
		29	29 4062	0056
Total		185	185 0000	0086
4	71604	282	286. 5422	. 0720
		62	59 9156	0725
		78	75 5422	0800
Total		422	422 0000	2245

Mather (*13*) has suggested that the goodness of fit test may be made more sensitive by basing the formulation of the expected phenotypic frequencies of each group of segregates upon the respective recombination fraction of the individual group, determining the total χ^2 value, and comparing the value with the χ^2 calculated upon the basis of the joint estimate of the recombination fraction. In table 8 the goodness of fit test, based upon the agreement between the observed F_2 and F_3 frequencies of the individual families and the expected frequencies, assuming the recombination fraction of the respective family, is given. The χ^2 values for the four families are 0.0161, 0.8494, 0.0086, and 0.2245. Subtraction of the sum of these values from the total χ^2 calculated on the basis of the recombination fraction of the combined populations (table 6), results in the χ^2

3.1559. This difference is due to discrepancies in the recombination fractions shown by the four families and has three degrees of freedom, the probability value being 0.38. The four families agree in the recombination fraction that they show.

Similarly, the more sensitive test may be applied in determining the significance of the discrepancies in the recombination fractions shown by the F_2 and F_3 segregations. The goodness of fit test, based upon the agreement between the observed phenotypic frequencies of the individual generations and the expected frequencies, assuming the recombination fraction of the respective generation, is given in table 9. The χ^2 values for the F_2 and F_3 segregations are 0.6462 and 0.0054, respectively. Subtraction of the sum of these values from the χ^2 calculated on the basis of the recombination fraction of the combined population (table 7), results in the χ^2 value 0.9328. Since this remainder is concerned with the difference between the two segregations, there is one degree of freedom, and the probability value is 0.35. It is concluded that the two sets of data do not deviate significantly from the linkage value (18.5 percent).

TABLE 9.— *Goodness of fit test, based upon the agreement between the observed phenotypic frequencies of the individual generation and the expected frequencies, assuming the recombination fraction of the respective generation*

| Generation | $P = (1-p)^2$ | Frequencies | | $\frac{(O-C)^2}{C}$ |
		Observed	Calculated	
F_2	.67907	441	450.0838	0.1833
		110	107.8324	.0436
		121	114.0838	.4193
Total		672	672.0000	.6462
F_3	.63355	218	218.5847	.0016
		61	60.8306	.0005
		53	52.5847	.0033
Total		332	332.0000	.0054

BACKCROSS POPULATION

The cross-over value for the small backcross progeny ($r_1^{ro} cl/R_1^{RO} Cl \times r_1^{ro} cl/r_1^{ro} cl$) has been shown to be 17.143 percent. The agreement of the observed backcross phenotypic-class frequencies with the calculated frequencies, based upon the percentage of recombinations, is shown in table 10. The expectations of the four phenotypic classes were determined by the following formulas:

$$R_1^{RO} Cl = \frac{n}{2}(1-p)$$

$$R_1^{RO} cl = \frac{n}{2}(p)$$

$$r_1^{ro} Cl = \frac{n}{2}(p)$$

$$r_1^{ro} cl = \frac{n}{2}(1-p)$$

where p equals the recombination fraction. The χ^2 value is 1.908, which corresponds to a probability value of 0.40, since there are two degrees of freedom, one degree having been lost in the estimation of linkage.

TABLE 10.—*Goodness of fit test, based upon the agreement between the observed phenotypic frequencies of the backcross* $(r_1{}^{ro}cl/R_1{}^{RO}Cl \times r_1{}^{ro}cl/r_1{}^{ro}cl)$ *and the expected frequencies, assuming the recombination fraction of the backcross segregates* $(p = 0.17143)$

Phenotypic class	Frequencies		x^2
	Observed	Calculated	
$R_1{}^{RO}Cl$	52	58	0.6207
$R_1{}^{RO}cl$	10	12	.3333
$r_1{}^{ro}Cl$	14	12	.3333
$r_1{}^{ro}cl$	04	58	.6207
Total	140	140	[1] 1.9080

[1] Degrees of freedom = 2, probability = 0.40.

The cross-over value (17.1 percent) obtained from the backcross data differs from the value (18.5 percent) obtained in the F_2 and F_3 segregation of the four families. In table 11 the goodness of fit test, based upon the agreement of the observed phenotypic frequencies of the backcross and the expected frequencies, assuming the recombination percentage 18.57 of the total population of F_2 and F_3 segregates of the four families, is given. The observed and expected frequencies agree satisfactorily, the probability value being 0.5. It is assumed that the cross-over value of the backcross population does not differ greatly from the value (18.5 percent) determined from the 1,004 F_2 and F_3 segregates of the four families.

TABLE 11.—*Goodness of fit test, based upon the agreement between the observed phenotypic frequencies of the backcross* $(r_1{}^{ro}cl/R_1{}^{RO}Cl \times r_1{}^{ro}cl/r_1{}^{ro}cl)$ *and the expected frequencies, assuming the recombination fraction of the total population of the* F_2 *and* F_3 *segregates of the four families* $(p = 0.18499)$

Phenotypic class	Frequencies		x^2
	Observed	Calculated	
$R_1{}^{RO}Cl$	52	57.0507	0.4471
$R_1{}^{RO}cl$	10	12.9493	.6717
$r_1{}^{ro}Cl$	14	12.9493	.0853
$r_1{}^{ro}cl$	64	57.0507	.8465
Total	140	140.0000	[1] 2.0506

[1] Degrees of freedom = 3, probability = 0.57.

SUMMARY AND CONCLUSIONS

Crosses were made between two strains of upland cotton characterized respectively by red plant, noncluster habit $(R_1{}^{RO}R_1{}^{RO}Cl\ Cl)$ and green plant, cluster habit $(r_1{}^{ro}\ r_1{}^{ro}\ cl\ cl)$.

Although the F_1 differed slightly from the noncluster parent, the noncluster characteristic is dominant, or practically so.

In the F_2 and backcross generations, good 3 : 1 and 1 : 1 ratios of noncluster to cluster were obtained, verifying the previous conclusion that the characteristic is controlled by one genetic-factor pair.

Detailed studies in linkage detection and linkage estimation show that the $R_1{}^{RO}$ and Cl genes belong to the same linkage group and that the percentage of recombinations under the conditions of this study is not far from 18.5.

These results verify the previous reports regarding the existence of the linkage of the two genes. However, the recombination fraction determined in these studies is somewhat higher than that previously reported.

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INHERITANCE OF A LEAF VARIEGATION IN BEANS [1]

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INTRODUCTION

In the course of investigations for the development of mosaic resistance in beans (*Phaseolus vulgaris* L.), a leaf variegation was frequently observed in progenies from hybrids in which Corbett Refugee, a selection from Stringless Green Refugee, had been used as one of the parents and also in progenies from hybrids involving other parental stock. The symptoms, which somewhat resembled those caused by a virus, differed from those of the common bean mosaic, and repeated attempts to infect susceptible bean varieties by the usual methods proved that this abnormality was not of a virus nature. It has been demonstrated by a study of these hybrids that this variegation is heritable (7).[3]

Other heritable leaf abnormalities and chlorophyll-deficient types in beans have been described by Burkholder and Muller (1), Parker (3, 4), Johannsen (2), and Tjebbes and Kooiman (6).

In 1936 Harrison and Burkholder,[4] and some time later Horsfall, Burkholder, and Reinking,[5] reported in Wisconsin Refugee a disease which appeared to them to be a new virus disease of beans and which they called "one-sided mosaic". The symptoms described for this disease coincide almost exactly with the abnormality described in this paper.

In 1939 Reinking and Witham (5) found that in certain bean plantings in New York as many as 22 to 27 percent of variegated plants were present in Idaho Refugee and in Corbett Refugee, one of the parents of Idaho Refugee. The variegation was not identical in all respects with the condition described in this paper.

The writer has observed variegation in commercial plantings of Wisconsin Refugee and Idaho Refugee beans grown in Colorado and Idaho. Both varieties are of the same parentage. Although the percentage of affected plants was small in most cases, it was not difficult to find abnormal plants.

Two kinds of leaf variegation were encountered in these studies: (1) A type that appeared on the primary leaves and later on the trifoliate leaves if the plant survived, the symptoms on these leaves frequently being more severe than those on the simple leaves; and (2) a type with similar symptoms confined to the trifoliate leaves. This second type of variegation was most common under field conditions.

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The symptoms of these two types are impossible to differentiate on the trifoliate leaves. Plants with extremely variegated primary leaves usually die. In some cases the symptoms of the type confined to the trifoliate leaves are not noted until the plant has reached considerable size. When these studies were initiated it was assumed that such symptoms noted only on the trifoliate leaves were a delayed expression of the variegation that also appeared on the primary leaves. It has since been determined that the primary-leaf variegation is inherited independently of the type that occurs only on the trifoliate leaves and is governed by different genetic factors. These studies deal only with the inheritance of the leaf variegation that appears on the primary leaves of seedling plants. The data presented are based on results of crosses between Corbett Refugee and several other varieties of beans and of other crosses between variegated and normal green plants.

MATERIAL AND METHODS

The crosses involved in this study are listed in table 1. The hybrids tested in 1937 and 1938 were not made for the purpose of studying the inheritance of variegation; they were made for breeding disease-resistant varieties. The variegation character was not known at that time, and hence the exact nature of all the plants used in the crosses with regard to variegation was not recorded. The symptoms of variegation in Corbett Refugee are in most instances very mild and are readily overlooked. The strain of Corbett Refugee used in the crosses made in 1937 was of this kind, and the variegation was not detected. No record was made of the presence of variegation in the strains of Corbett Refugee used in the crosses tested in 1938, but it may be assumed that no plants showing any unusual abnormality would have been used in hybridization. The Corbett Refugee and the unnamed varieties of which one of the parents was Corbett Refugee, used in the crosses tested in 1939 varied; in some plants the symptoms of variegation were not observed, whereas in others variegation was present in different degrees (see table 1). When selfed, progenies from the parents contributing the factors for variegation produced only a small percentage of variegated plants (see table 2), whereas those from the green parents bred true for the normal green condition.

The crosses (see table 1) were made in the greenhouse at the United States Horticultural Station at Beltsville, Md. The F_1 hybrids were grown under field conditions at Greeley, Colo., and remained free from the common bean mosaic and other virus diseases. Although it is known that natural field hybridization may occur in beans, this was not detected from a study of other characters, and hence it in no way accounted for the deviations which are noted in the segregating generations. The seed of each plant was harvested separately and used later for a study of the F_2 generation. These progenies were grown in the greenhouse, the temperature of which was maintained at from 70° to 80° F., and the plants were classified as soon as the simple leaves were fully developed. Certain F_2 plants were chosen at random within the normal-appearing and variegated classes and grown to maturity to give rise to the F_3 families. The F_3 families were grown both under field conditions and in the greenhouse and were classified in the seedling stage to obtain the F_2 genotypic distribution.

The data were subjected to the χ^2 test for goodness of fit to certain theoretical ratios. This test was applied to F_2 families independently and to F_3 families from F_2 plants.

SYMPTOMS

The symptoms of the leaf variegation described herein always appear on the primary leaves (fig. 1, *G–I*). They may vary considerably, with some leaves practically devoid of the normal green pigmentation (fig. 1, *H*) and with only small islands of light-green tissue interspersed with yellow. In some cases the leaf is almost entirely light-yellowish green in color with small islands of darker green tissue (fig. 1, *II*), whereas in others small islands of dark green may be surrounded by deep-yellow-colored tissue. Occasionally a leaf may show over a small portion of its surface only a slight chlorotic streaking or mottling, which may be readily overlooked. Leaves that are mostly devoid of the green pigment are smaller than those with more chlorophyll, and they usually die (fig. 1, *H*). It is probable that such plants would be overlooked under field conditions unless examinations were made early in their growth.

Symptoms on the trifoliate leaves also are variable. Sectoring is common, with only small portions of the leaf yellow and the remaining portion normal green (fig. 1, *A*, *C*). Not infrequently only a few islands of yellow are noticeable. A very characteristic symptom on plants that have not died in the seedling stage is a variegation of only one side of the leaflet (fig. 1, *A*). Symptoms of this kind were pointed out by Harrison and Burkholder[6] and by Horsfall et al.[7] The unaffected portion grows normally, whereas the chlorotic half does not. Sometimes growth almost completely stops on the chlorotic side of the leaflet; thus, the leaflet becomes decidedly distorted and curls toward the affected portion (fig. 1, *A*). This peculiar symptom suggested the name "one-sided mosaic" to Horsfall et al.[7]

A necrotic streaking or spotting may occur on the petiole and affected parts of the leaflet. The trifoliate leaflets that arise from the necrotic side of the petiole may be affected and only one or sometimes two of the leaflets remain normal (fig. 1, *C*). In like manner, only a portion of the plant may be so affected.

Seriously variegated plants that do not die in the seedling stage sometimes become somewhat rosetted and considerably stunted. The internodes are much shorter than those of normal plants, and adventitious buds commonly arise in the axils of the leaves. Affected plants grown under ideal conditions may sometimes attain maturity and produce a few curled and distorted pods. Plants mildly affected may produce a normal seed crop.

The pods on extremely variegated plants are distorted, frequently curled, and abnormal in shape, size, and color (fig. 1, *D–F*). They sometimes produce seeds that, although appearing normal in most respects, are subnormal in size.

[6] See footnote 4.
[7] See footnote 5.

FIGURE 1.–Symptoms of variegation on bean: *A*, F_2 hybrid (Corbett Refugee ×
Stringless Green Refugee) showing malformation of one side of certain tri-
foliate leaflets; *B*, trifoliate leaf of another hybrid of same cross with extreme
malformation and dwarfing of one leaflet; *C*, trifoliate leaf with only two of the
three leaflets showing the variegation; *D–F*, pod malformation due to variega-
tion; *G–I*, variegated primary leaves of Wisconsin Refugee.

EXPERIMENTAL RESULTS

PARENTS

As mentioned previously, variegation in Corbett Refugee was in most instances very mild and was readily overlooked. Although in some instances it was quite evident, it was seldom as severe as that in the segregating generations. In no case did any of the strains of Corbett Refugee or other variegated parents used in the crosses (table 1) breed true for variegation. This fact tends to explain certain results discussed later. The breeding behavior of a number of variegated parents when selfed is recorded in table 2, where it is seen that only a small percentage of their offspring manifested variegation.

TABLE 1.— *F_2 segregations from crosses between Corbett Refugee or other strains or varieties contributing the genes controlling variegation and normal green varieties*

Parentage of crosses	Nature of parent contributing variegation	Year tested	Number of F_2 plants that were		x^2 for 15 : 1 ratio [1]
			Green	Variegated	
U. S. No. 1 Refugee × Corbett Refugee	Normal appearing	1937	390	28	0.147
Corbett Refugee × U. S. No. 1 Refugee	do	1937	596	52	4.962
Corbett Refugee × Stringless Green Refugee	do	1937	171	4	4.658
Corbett Refugee × Perry Marrow	Unknown	1938	1,502	85	2.169
Perry Marrow × Corbett Refugee	do	1938	308	19	.108
Geneva Red Kidney × Corbett Refugee	do	1938	1,055	62	.930
Corbett Refugee × Geneva Red Kidney	do	1938	713	29	6.960
Canadian Wonder Bush × Corbett Refugee	do	1938	418	27	.025
Bountiful × Corbett Refugee	Normal appearing	1938	228	23	3.620
Small White × Corbett Refugee	Unknown	1938	332	17	1.127
Unnamed variety (9D)[2] × Blue Lake	Seriously variegated	1939–40	860	39	5.615
Unnamed variety (10A)[2] × Small White	Mildly variegated	1939–40	276	9	4.641
Wisconsin Refugee [3] × Red Kidney	Seriously variegated	1939–40	201	15	.177
Small White × Corbett Refugee	Normal appearing	1939–40	830	47	1.184
Red Kidney × Corbett Refugee	Moderately variegated	1939–40	410	29	.089
Corbett Refugee × Red Kidney	do	1939–40	116	5	.948
Perry Marrow [4] × Great Northern	Unknown	1938	226	15	.001
Cranberry [4] × Pinto	Normal appearing	1938	338	32	3.658
Unnamed variety [4] × No. 780 [5]	Variegated	1938	175	10	.245
Red Kidney [4] × Blue Lake	Normal appearing	1939–40	305	16	.892

[1] 5-percent point = 3.841.   [2] Corbett Refugee, one of parents   [3] Variegated parent
[4] Parent contributing genes controlling variegation.   [5] Number carried in file of writer

TABLE 2.   *Breeding behavior of a number of the selfed variegated parents used in the several crosses*

Variegated parent	Strain No. [1]	Number of plants that were		
		Normal appearing	Variegated	Doubtful
Corbett Refugee	473-B	801	18	0
Do.	473-5 B	781	22	25
Do.	473-7-B	1,140	12	2
Do.	8C	503	3	0
Do.	5D	423	4	0
Wisconsin Refugee	6A	429	2	0
Unnamed variety	10A	422	10	0
Do.	9D	342	2	0

[1] Numbers carried in files of writer.

F_1 GENERATION

The F_1 plants from reciprocal crosses between Corbett Refugee, as well as certain unnamed varieties, and normal green varieties were all normal green, which indicated that the factor or factors for variegation were recessive to normal green in inheritance. In some cases the Corbett Refugee parent was variegated; in others it was not. The nature of the parents used in the crosses is shown in table 1.

Reciprocal crosses were not made with every hybrid combination, but where they were made and tested they behaved about alike (table 1). This proved that the inheritance was not cytoplasmic.

F_2 GENERATION

From 20 progenies comprising 40 crosses, 9,983 F_2 plants were grown, a part of these being produced in the greenhouse and the others in the field. Fifteen of the twenty progenies showed satisfactory fits to a 15:1 ratio, as is seen in table 1. The other progenies showed significant deviations from this ratio, but only 1 was highly significant. Thirteen of the progenies had deficiencies of variegated recessives.

Although differences in the intensity of the variegation were observed, the plants were not classified as to degree of severity but were recorded only as variegated. Environment, without doubt, influenced the expression of the character, but the wide variation from a mild mottling to extreme malformation could not be reasonably accounted for on the basis of environmental factors alone, since various degrees of variegation occurred simultaneously.

F_3 GENERATION

In the F_3 generation no family of less than 66 plants was used in the analysis.

F_3 progenies were grown from 204 normal green F_2 plants that were derived from 18 original crosses. Of these, 89 progenies bred true for normal green, 61 segregated into 15 green to 1 variegated plant, and 54 segregated into 3 green to 1 variegated plant, which approximated a 7:4:4 ratio, respectively, with a χ^2 value of 1.207.[8] This substantiated the duplicate-factor hypothesis as found in the F_2 generation. Of the 61 progenies that segregated into a 15:1 ratio, 26 were deficient in variegated plants. Among the families that showed a 3:1 segregation, practically every one showed this deficiency.

DOUBLE RECESSIVE CLASS

Several hundred F_2 variegated seedlings were transplanted in order that they might be grown to maturity, but because of the lack of chlorophyll only a small percentage survived. At the beginning of the study the number of transplanted variegated seedlings was not recorded since it was not assumed that they were lethal, and as a result the exact percentage of plants that survived could not be calculated. Later studies, however, showed that from 849 transplanted seedlings derived from 136 F_3 progenies, only 119 plants or 14.0 percent survived and those plants that grew to maturity produced a relatively small number of seeds. In another experiment, 35 variegated seedlings derived from 4 F_2 progenies were transplanted. Only 11 of them survived, and these produced 105 seeds.

It is seen from table 3 that from a total of 139 variegated progenies that survived and were grown in the next generation, only 23 produced all variegated plants. There were only 83 plants produced from these

[8] 5-percent point=5.991.

23 progenies, or an average of less than 4 plants per progeny. It is probable that if the populations had been larger all progenies would have produced some green plants. Among the 37 progenies that produced all green plants, the individual populations were likewise too small to permit definite conclusions. The 79 progenies that produced 230 variegated and 427 green plants likewise had small individual populations. It is probable that if the F_2 variegated plants had produced fairly large progenies all of them would have contained both green and variegated plants.

TABLE 3.— *Breeding behavior of variegated F_2 plants in the F_3 generation*

F_3 segregation	Number of F_3 progenies	Number of F_3 plants that were	
		Green	Variegated
All green plants	37	124	0
Green and variegated plants	79	427	230
All variegated plants	23	0	83
Total	139	551	313

Since the variegated parents used in the crosses did not breed true for variegation and since the segregating progenies in the F_2 and F_3 generations were deficient in variegated plants, it should not be expected that the double recessive lines would breed true for variegation. As mentioned previously, the progenies that produced all variegated plants were of such small populations that the data appear hardly conclusive. In general the data corroborate those of the parental material as well as those of many of the segregating progenies in that a great number showed deficiencies of variegated plants.

DISCUSSION

The results of crossing Corbett Refugee, as well as several other strains and varieties of beans that carry the factors for variegation, with normal green plants indicate that this condition is heritable and is governed by two major Mendelian factors behaving as recessives. It is evident that the data do not conform in every detail to the hypothesis of duplicate factors.

In spite of the fact that it was shown that the Corbett Refugee parents produced only a relatively few variegated offspring (table 2), it is believed that they were homozygous recessive for both variegated genes. The fact that the expression of variegation was much more pronounced in the F_2 segregates than in Corbett Refugee suggests that one or several inhibiting factors are carried by Corbett Refugee. The belief that such modifying factors exist is further strengthened by the deficiency of recessives in the F_2 generation and in most of the F_3 segregating families and also by the failure of many of the variegated recessives to breed true.

As mentioned earlier, a high percentage of the variegated plants were lethal, and it is assumed that these are the true-breeding recessives. Those that survived and produced seed were probably the ones carrying some of the inhibiting factors, possibly in the heterozygous condition. In the progeny of these plants the variegated

character may again have been suppressed, the action of the inhibitors accounting for the high percentage of green plants in the progeny of the recessive class.

This heritable leaf variegation has not been observed in many bean varieties that are grown commercially, but it has been of importance in the Wisconsin Refugee variety and to a lesser degree in the Idaho Refugee. Both of these varieties have Corbett Refugee as one of the parents. This variety has been used extensively in hybridization because of its immunity to the common bean mosaic virus. In practically all cases where it was used, the progenies exhibited variegation. Although there may be strains of Corbett Refugee that are free from this condition, it should be used as a parent only with caution. This weakness should be recognized and watched for carefully. Since inhibiting factors may suppress the expression of the variegated symptoms, normal-appearing strains may actually carry the factors for this condition and when outcrossed with normal green varieties the variegation would be expressed. If a mosaic-resistant Refugee type bean is desired as a parent, it is suggested that the U. S. No. 5 Refugee had better be used. This variety, although derived from a cross with Corbett Refugee, is free from variegation and is resistant to the common bean mosaic.

SUMMARY

Two types of leaf variegation have been observed in beans, one of which appears both on the primary and the trifoliate leaves and the other only on the trifoliate leaves. The symptoms of the two are similar, but they are inherited differently. The inheritance of the first is described in this paper.

The results obtained when plants of Corbett Refugee and other varieties that carry the factors for variegation were crossed with normal green plants of several varieties are presented. For the most part the data support the two-factor Mendelian hypothesis. The F_1 plants of reciprocal crosses were normal green in appearance, and a ratio of 15 green plants to 1 variegated was obtained in the F_2 generation.

In the F_3 generation from green F_2 plants, a 7:4:4 ratio of all green, 15 green to 1 variegated, and 3 green to 1 variegated was obtained.

The variegated recessive progenies, except in a few instances where the populations were small, did not breed true in the F_3 generation. It is assumed that this lack of true breeding was due to one or several inhibiting factors that suppressed the variegation character. The death of a high percentage of the variegated plants was probably due to the absence of these inhibitors.

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ANAEROBIC DECOMPOSITION OF WHEAT STRAW BY THERMOPHILES AND THE QUANTITY OF GAS PRODUCED [1]

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INTRODUCTION

This contribution is a continuation of the series (*3, 4, 5,*) [3] on the changes in fibrous farm wastes caused by anaerobic microbial fermentation and the significance of these changes in the possible utilization of such materials for the production of fuel gas, pulp, and fertilizer. The first paper (*3*) dealt specifically with the quantity and quality of fuel gas that could be produced from these various wastes, particularly cornstalks, wheat straw, artichoke tops, and seed flax straw. It was shown that the rate of gas production by the anaerobic fermentation of farm wastes was greater at 50° to 55° C. than at 28° to 30°, although the total amount of gas produced was about the same. The degree of fineness of the material and possibly its internal structure played an important part in its susceptibility to decomposition. Periodic chemical analyses of the residues were not made, since the main objective was to determine how much utilizable gas could be produced from a unit amount of waste material.

The next logical step was to ascertain at what rate the individual components of the wastes were decomposed, because this information should be of value in planning the preliminary treatment of these wastes in the preparation of pulp, in retting, and in composting. The authors had found that at 28° to 30° C. (*4*) and at 50° to 55° (*5*) cornstalk flour was fermented more rapidly than chopped cornstalks and produced more gas in a given unit of time; in cornstalk flour the losses of cellulose and pentosans were considerably greater, and the loss of lignin less, than in the chopped cornstalks. The loss of lignin in the cornstalk flour was much less than the loss of either the cellulose or the pentosans.

The study reported in this paper was undertaken to determine the decomposition of the various constituents of wheat straw by a thermophilic anaerobic fermentation and the quantity of gas produced.

PROCEDURE

The test materials were prepared by grinding wheat straw in a hammer mill. The material ground fine enough to pass a circular

opening 1 mm. in diameter is called straw flour. The term "chopped straw" is used to designate the material composed wholly of coarse particles about one-fourth of an inch in length.

The thermophilic methane-producing seed was originally developed from sewage sludge, as described in a previous publication (*3*). Before it was used it was passed through a screen with circular openings 1 mm. in diameter. All solids remaining on the screen were discarded. The seed was analyzed, and sufficient urea was added (0.90 gm. per liter) to bring the ammonia content of the mixture to from 500 to 800 parts per million.

The fermentation apparatus consisted of a 2-quart mason jar into the neck of which was inserted a rubber stopper provided with three tubes for (1) the removal of samples for pH determination, (2) the addition of chemicals, and (3) the collection of gas. The rubber stopper was held in position by means of the metallic mason-jar lid, the center of which was cut out to permit protrusion of the tube outlets. Details of construction of the fermentation, gas-collection, and gas-measurement apparatus have been described by the authors in previous publications (*3, 4*).

The fermentation mixtures consisted of 30 gm. of the wheat straw and 1 liter of the seed. At the beginning of the experiment, hydrated lime was added (equivalent to 1 gm. of CaO per liter of mixture) to adjust the mixture to the desired pH. The mixtures were incubated at 50° to 55° C. for 20 days, and analyses were made periodically.

At the end of each fermentation period the mixtures were removed from the incubator and treated as follows: All the straw-flour mixtures were evaporated to dryness on the steam hot plate, ground to pass a 30-mesh screen, and analyzed. In the chopped-wheat-straw mixture it was necessary to separate the seed from the straw in order to ascertain the actual loss of constituents of the straw. The seed was therefore removed with a screen having round holes 1 mm. in diameter. The straw fraction retained on the screen was washed several times with distilled water, a total of 1,000 cc. being used. The washed straw was then dried on the steam hot plate, ground to pass a 30-mesh screen, and analyzed. The sludge fraction (the portion that passed through the screen) and the washings from the straw fraction were combined, evaporated to dryness, ground to pass a 30-mesh screen, and analyzed.

The following determinations were made on the dried residues: (1) Total solids; (2) volatile solids (loss on ignition at about 600° C. for 1 hour) by the American Public Health Association method (*1*); (3) pentosans by the Association of Official Agricultural Chemists' method (*2*); (4) cellulose by the Norman and Jenkins' method (*6*) (with corrections made for ash and pentosans); (5) alpha cellulose as outlined by Schorger (*9*); and (6) lignin by the Norman and Jenkins' method (*7*) with the substitution of a 2-percent-acid hydrolysis for 2 hours for the 5-percent hydrolysis normally used. Duplicate lignin samples were taken. One was ashed, and the other was analyzed for Kjeldahl nitrogen by the American Public Health Association method (*1*). A correction for ash was made in calculating the results, but no correction for nitrogen was made, inasmuch as Norman and Jenkins (*8*) have shown that the usual protein factor is not reliable.

Gas was collected from the following fermentations: (1) Straw flour plus active methane-producing seed; (2) chopped straw plus seed;

(3) seed (control); (4) straw flour plus water (control); and (5) chopped straw plus water (control). The gas produced each day was measured, and its composition was determined periodically. The pH was ascertained daily.

DISCUSSION OF RESULTS

DECOMPOSITION OF CONSTITUENTS

The quantities of the various constituents in the straw flour, in the chopped straw, and in the seed, before fermentation, are shown in table 1. Table 2 shows the decomposition of constituents in the seed control. Most of the changes (losses or increases) noted in the constituents in the seed control are very small and probably are within the experimental error of the methods of analysis and of the procedure used in this study. These small changes are not considered significant.

TABLE 1.— *Constituents in wheat-straw-flour and chopped-wheat-straw mixtures before fermentation*

Constituents		Wheat-straw-flour mixture			Chopped-wheat-straw mixture		
		Seed	Wheat-straw flour	Total	Seed	Chopped wheat straw	Total
Total solids	grams	15 5	28 4	¹ 45 7	15.5	28 4	¹ 45 7
Volatile solids	do	10 4	26 4	² 37 7	10 4	27 0	² 38.3
Pentosans	do	.5	8 3	8 8	5	8 5	9 0
Crude cellulose	do	6	15 5	16 1	6	15.1	15.7
Pentosans in the cellulose	do	1	5 2	5 3	.1	4 7	4 8
Cellulose (corrected) ³	do	5	10 3	10.8	5	10.4	10 9
Alpha cellulose	do		10 2			9 8	
Lignin	do	5 6	4 3	9.9	5 6	4 4	10 0
Kjeldahl nitrogen in the lignin. percent		2 2	5		2 2	1.0	

¹ Includes 0 90 gm of urea an 1 1 0 gm of CaO
² Inclu les 0 90 gm of urea
³ Corrected for ash and pentosans

Data on the break-down of the various constituents in the straw-flour fermentation are recorded in table 3, and similar data for the chopped straw are recorded in table 4. The results are presented graphically in figure 1. The values shown in table 4 were obtained from tables 5 and 6 by taking the sum of the constituents of the straw fraction and of the sludge fraction of the chopped-straw mixture. The percentage losses of the various constituents, listed in tables 3 and 4, were computed after corrections were made for losses in the seed (table 2).

Throughout the straw-flour fermentation, the percentage losses of cellulose and pentosans, which were nearly the same, were rather high. The cellulose was attacked as readily as the pentosans, and as the losses were high the residue would probably be of little value for making paper pulp. The behavior of these constituents was different from that in the fermentation of cornstalks (5), where the loss of pentosans during the earlier stages of the fermentation was usually greater than that of cellulose.

In the chopped-straw fermentations, the percentage losses of cellulose and pentosans were about the same during the entire fermentation period. In this material, also, the high loss of cellulose would be unfavorable for a fermentation paper-pulping process.

TABLE 2.—*Decomposition of constituents in the seed control and quantity of gas produced after fermentation for various periods*

Time fermented (days)	Quantity present								Quantity decomposed [1]						Gas produced [2]		
	Total solids	Volatile solids	Pentosans	Crude cellulose	Pentosans in the cellulose (corrected) [3]	Cellulose (corrected) [3]	Lignin	Kjeldahl nitrogen in the lignin	Total solids	Volatile solids	Pentosans	Pentosans in the cellulose (corrected)	Cellulose (corrected)	Lignin	Daily	Cumulative total	Per gram of volatile solids lost
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Cubic centimeter*	*Cubic centimeter*	*Cubic centimeter*
0	15.5	10.4	0.5	0.6	0.1	0.5	5.6	2.2	0.0	1.4	0	0	0.0	1.8	45	45	322
1	15.3	10.3	.5	.6	.1	.6	5.5		2.1	2.2	0	0	+20.0	1.8	0	45	196
2	15.2	10.2	.5	.6	.1	.6	5.5		3.2	3.4	0	0	+20.0	3.6	0	45	128
3	14.6	10.2	.5	.7	.1	.6	5.4		6.0	5.4	0	0	+20.0	5.4	0	45	80
5	14.7	9.8	.5	.7	.1	.7	5.1	1.3	11.7	10.2	20.0	0	+40.0	8.9	0	45	42
10	13.7	9.3	.4		.1												
20	13.1	8.8	.4	1.0	.1	.9	4.9	1.6	15.5	15.1	20.0	0	+90.0	12.5	0	45	29

[1] Plus sign indicates a gain   [2] Corrected to 30 inches of mercury and 60° F   [3] Corrected for ash and pentosans.

TABLE 3.—*Decomposition of constituents in the wheat-straw-flour mixture (straw flour + seed) and quantity of gas produced after fermentation for various periods*

Time fermented (days)	Quantity in mixture									Decomposition of constituents in straw [1]						Gas produced [2] per gram of—			Total gas [2] produced
	Total solids	Volatile solids	Pentosans	Crude cellulose	Pentosans in the cellulose (corrected) [3]	Cellulose (corrected) [3]	Alpha cellulose	Lignin	Kjeldahl nitrogen in the lignin	Total solids	Volatile solids	Pentosans	Pentosans in the cellulose	Cellulose (corrected) [3]	Lignin	Wheat-straw flour	Volatile solids in straw	Volatile solids lost	
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Cubic centimeter*	*Cubic centimeter*	*Cubic centimeter*	*Cubic centimeter*
0	45.7	37.7	8.8	16.1	3.3	10.9	10.2	9.9											
1	34.6	29.4	6.6		3.3	10.9		8.9	1.9	38.1	31.1	26.5	36.5	30.1	20.9	30.5	32.8	105	965
2	33.1	27.5	6.1	10.5	3.2	7.3	7.2	8.7	2.3	43.3	37.9	32.5	40.4	35.0	25.6	87.9	94.5	250	2,495
3	32.5	26.3	5.4	8.1		5.0		8.7	2.4	44.7	41.7	41.8	55.6	51.5	30.2	140.0	150.6	361	3,975
5	31.1	25.1	4.5	7.5	2.9	5.4	5.6	8.3	2.4	48.2	45.5	51.8	59.6	53.4	20.9	188.4	202.7	416	5,330
10	26.6	19.8	2.3	3.2		2.3		8.3	2.6	60.9	63.6	77.1	84.6	83.5	25.6	320.6	344.9	542	9,105
20	23.1	17.3	1.8	2.5	.7	1.8		7.6	1.9	71.1	71.2	83.1	88.5	88.3	37.2	375.7	404.2	568	10,670

[1] All figures in this section were computed after making corrections for losses in the seed (table 2)   [3] Corrected for ash and pentosans.
[2] Corrected to 30 inches of mercury and 60° F.   [4] For composition of original mixture see table 1.

TABLE 4.—*Decomposition of constituents in the chopped-wheat-straw mixture (straw + seed) and quantity of gas produced after fermentation for various periods*

Time fermented (days)	Quantity in mixture								Decomposition[1] of constituents in straw[2]							Gas produced[3] per gram of—			Total gas[3] produced
	Total solids	Vola-tile solids	Pento-sans	Crude cellu-lose	Pento-sans in the cel-lulose	Cellu-lose (cor-rected)[4]	Alpha cellu-lose	Lignin	Total solids	Vola-tile solids	Pento-sans	Pento-sans in the cel-lulose	Cellu-lose (cor-rected)[4]	Lignin	Kjel-dahl nitro-gen in the lignin	Chop-ped wheat straw	Vola-tile solids in straw	Vola-tile solids lost	
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Cubic centi-meters*	*Cubic centi-meters*	*Cubic centi-meters*	*Cubic centi-meters*
0 [5]	45.7	38.3	9.0	15.7	4.8	10.9	9.8	10.0											
1	41.2	35.5	9.4	15.8	4.7	11.0		9.0	15.5	10.0	7.1	0.2	+1.0	11.4		23.9	25.2	252	680
2	38.6	32.8	6.8	13.5	3.9	9.6		9.6	24.3	19.6	25.9	19.1	12.5	6.8		71.7	75.4	384	2,035
3	38.8	31.9	6.3	12.0	3.5	9.6		9.5	22.9	22.2	31.8	29.8	22.1	8.0		116.7	122.8	553	3,315
5	36.6	29.9	5.5	10.4	3.1	6.9		10.0	29.2	28.9	11.2	27.7	38.5	+6.8		178.2	187.4	649	5,060
10	32.9	26.2	4.7	7.7	2.1	5.6		9.6	39.1	40.7	49.4	57.4	51.0	+2.3		287.3	302.2	742	8,160
20	28.0	21.1	2.6	5.1	1.6	3.6		8.8	54.2	57.8	74.1	68.1	70.2	11.4		372.5	391.9	678	10,580

[1] Plus sign indicates a gain.
[2] All figures in this section were computed after making corrections for losses in the seed (table 2).
[3] Corrected to 30 inches of mercury and 60° F.
[4] Corrected for ash and pentosans.
[5] For composition of original mixture see table 1.

Cellulose and pentosans were decomposed much more rapidly in the straw flour that in the chopped straw throughout the entire fermentation period. The difference in break-down between the flour and the chopped material is interesting because practically no difference was noted in the total volume of gas produced from the flour and from the chopped straw (table 9). During the straw-flour fermentation some depressing action may have occurred that retarded the rate of gas production from the intermediate complexes formed during the break-down of the cellulose and the pentosans. This retarded rate of gas production might have been due to a difference in the nature of the break-down in the flour, but more likely it was due to the formation of an intermediate complex highly resistant to the production of
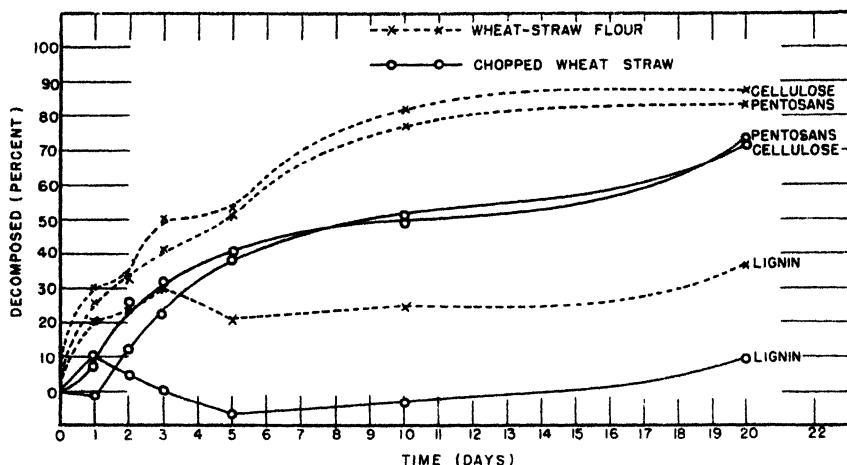
FIGURE 1.—Decomposition of the constituents of wheat straw in an active methane-producing seed at 50° to 55° C.

gas. However, further study is necessary to ascertain the reasons for this behavior.

The percentage losses of the pentosans associated with the cellulose, shown in tables 3 and 4 and graphically in figure 2, were practically the same as the corresponding percentage losses of cellulose in both the straw-flour and the chopped-straw fermentations.

Determination of alpha-cellulose was made when sufficient residue was available. These values, given in tables 3 and 4, are compared graphically with the Norman and Jenkins' cellulose (corrected for ash and pentosans) in figure 3. In both fermentations the values for alpha-cellulose agree fairly well with the corrected Norman and Jenkins' values for cellulose, and for practical purposes the corrected cellulose values may be considered as alpha-cellulose values in analyzing this type of material. This application is of considerable importance in the analysis of sludge samples in which the cellulose content is so low that sufficient material is not available for the actual alpha-cellulose determination.

In the chopped straw, the decomposition of lignin was very small. The lignin decomposed much more rapidly in straw flour, but even here it was more resistant to attack than were the cellulose and pentosans. In fact, during the earlier stages of the straw-flour fer-

mentation little decomposition was noted. Later, when most of the cellulose and pentosans had been decomposed, lignin was broken down at an appreciable rate.
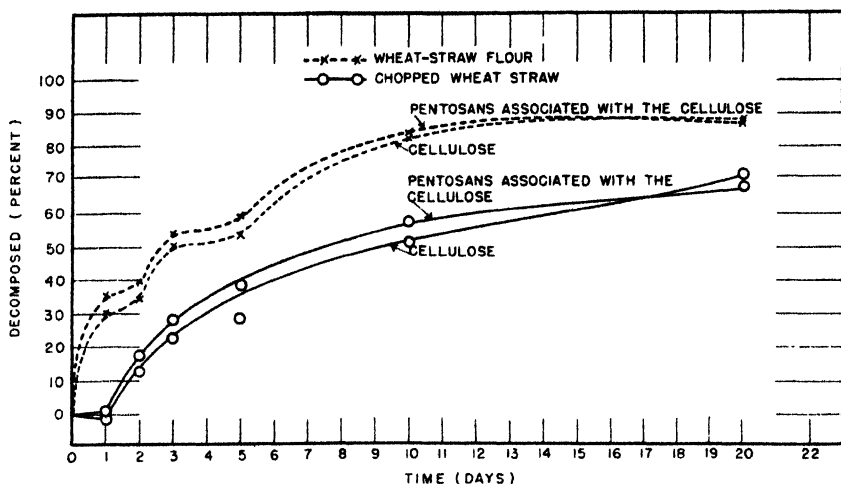
FIGURE 2.—Decomposition of the cellulose and pentosans associated with the cellulose in wheat straw in an active methane-producing seed at 50° to 55° C.
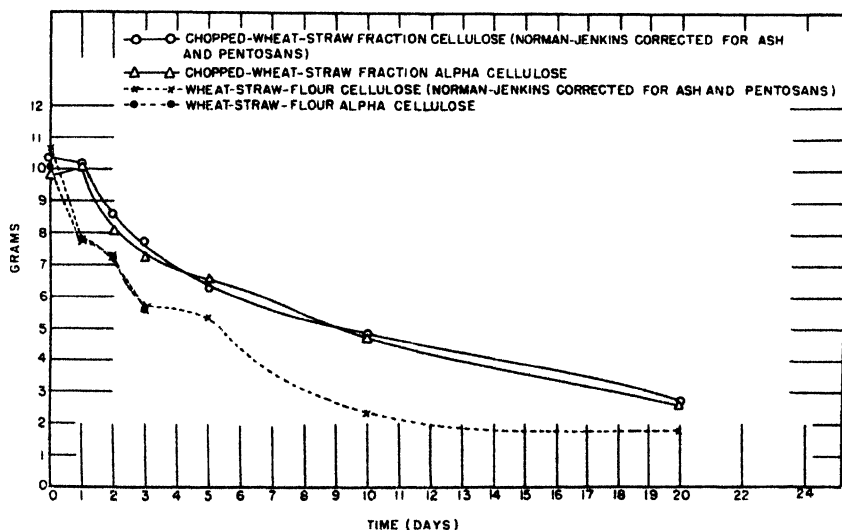
FIGURE 3.—Alpha cellulose and Norman and Jenkins' cellulose (corrected for ash and pentosans) in wheat straw in an active methane-producing seed at 50° to 55° C.

The behavior of lignin in both the straw-flour and the chopped-straw fermentation was rather striking. In both, decomposition of lignin was noted in the earlier stages of the fermentation, followed by an apparent increase and finally by a gradual decrease. In many cases this increase in lignin content was accompanied by an increase in Kjeldahl nitrogen in the lignin residue. This is shown graphically

in figure 4. Probably lignonitrogenous complexes were formed during the fermentation, especially during the earlier stages, and when the actual break-down was slight, these complexes increased the weight of apparent lignin (residue after the 72-percent-acid treatment). The real break-downs of lignin was probably greater than that shown by the anaylsis. Other investigators, Waksman and Iyer (*10*) and Norman and Jenkins (*8*) have noted this, and it has also been noted in earlier work in this laboratory (*4*). However, no definite conclusions can be drawn as to the actual decomposition of lignin because of the
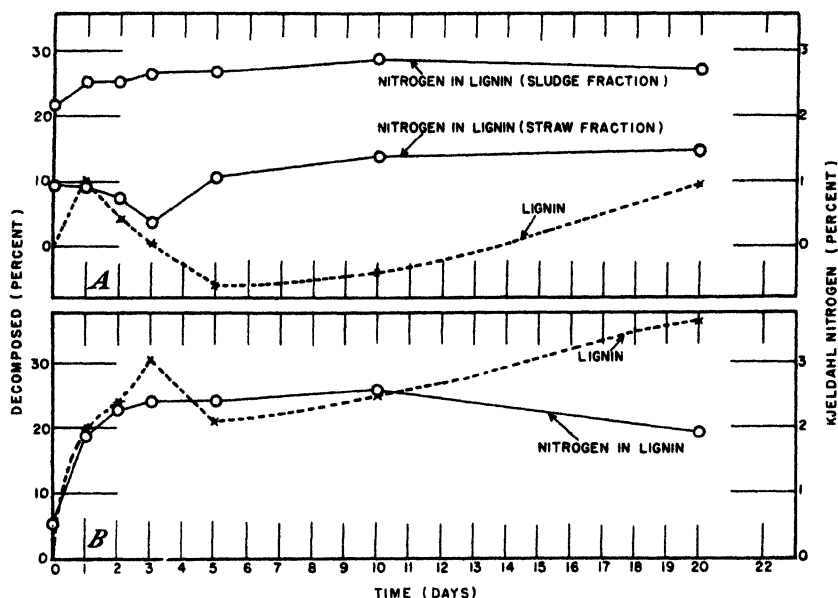
FIGURE 4.—Decomposition of lignin and the percentage of Kjeldahl nitrogen in the lignin residue in (*A*) chopped wheat straw and (*B*) wheat-straw flour in an active methane-producing seed at 50° to 55° C.

inadequacy of the present methods of analysis, as has been pointed out by Norman and Jenkins (*7, 8*).

In the straw-flour fermentation, in which appreciable decomposition of lignin occurred during the latter part of the fermentation, a distinct decrease was noted in the Kjeldahl nitrogen content of the lignin residue after 20 days' fermentation. This indicated that the lignoprotein complexes were undergoing decomposition. Apparently this attack on the lingoprotein complex was greater than that observed by Waksman and Iyer (*10*), who considered these complexes to be very resistant to microbial attack.

The break-down of the various constituents in the straw fraction from the chopped-straw fermentation is recorded in table 5, and that of the sludge fraction (material passing through a 1-mm. screen plus washings from the straw) in table 6. The results are presented graphically in figure 5.

TABLE 5.—*Quantity of constituents in the straw fraction of the chopped-wheat-straw mixture and the percentage removed after fermentation for various periods*

Time fermented (days)	Quantity present									Percentage removed					
	Total solids	Volatile solids	Pentosans	Crude cellulose	Pentosans in the cellulose	Cellulose[1] (corrected)	Alpha cellulose	Lignin	Kjeldahl nitrogen in the lignin	Total solids	Volatile solids	Pentosans	Pentosans in the cellulose	Cellulose[1] (corrected)	Lignin
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0[2]	28.4	27.0	8.5	15.1	4.7	10.4	9.8	4.4	1.0						
1	24.2	23.3	7.7	14.7	4.5	10.2	10.1	4.2	.9	14.8	13.7	9.4	4.3	1.9	4.5
2	21.1	20.3	6.1	12.4	3.8	9.6	9.0	3.8	.8	25.7	24.8	28.2	19.1	17.3	13.6
3	19.7	18.8	5.5	11.0	3.3	8.6	7.2	3.7	.4	30.6	30.4	35.3	31.8	25.0	15.9
5	17.2	16.5	4.9	9.6	3.0	7.8	6.6	3.6	1.1	39.4	38.9	42.4	29.8	40.4	18.2
10	13.1	12.7	4.2	6.8	2.0	6.2	4.7	3.2	1.4	52.8	53.0	50.6		52.9	27.3
20	8.2	7.7	2.0	4.0	1.1	2.8	2.6	2.1	1.5	71.1	71.5	76.5	76.6	73.1	52.3

[1] Corrected for ash and pentosans.
[2] For composition of original mixture see table 1
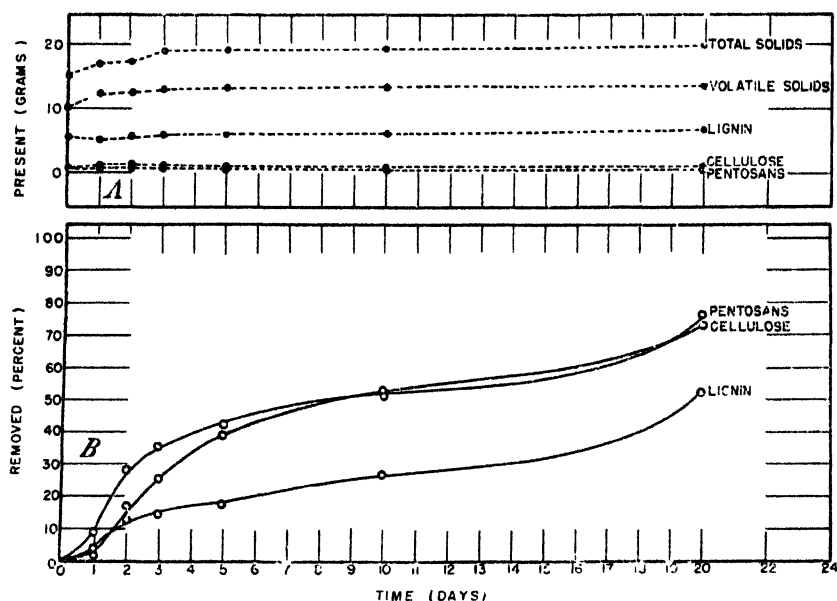
FIGURE 5.—*A*, The quantities of pentosans, cellulose, and lignin, together with the solids, present in the sludge fraction; *B*, percentage of pentosans, cellulose, and lignin removed from the straw fraction of chopped wheat straw in an active methane-producing seed at 50° to 55° C.

TABLE 6.—*Constituents in the sludge fraction of the chopped-wheat-straw mixture after fermentation for various periods*

Time fermented (days)	Total solids	Volatile solids	Pento- sans	Crude cellu- lose	Pento- sans in the cel- lulose	Cellu- lose [1] (cor- rected)	Lignin	Kjeldahl nitrogen in the lignin
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*
0 [2]	15.5	10 4	0 5	0 6	0 1	0 5	5 6	2.2
1	17.0	12 2	.7	1 1	2	9	5 2	2.5
2	17 5	12.5	.7	1.1	1	1 0	5 8	2.5
3	19 2	13 1	8	1 1	.2	8	6 0	2 7
5	19 3	13 3	6	8	1	.7	6.4	2.7
10	19 5	13 4	.6	8	1	.7	6 4	2.9
20	19 8	13 5	.6	1.2	.5	.7	6 7	2.7

[1] Corrected for ash and pentosans.
[2] For composition of original mixture see table 1.

The break-down of the pentosans and cellulose in the straw fraction occurred at about the same rate as in the mixture, indicating that these constituents had not accumulated in the sludge fraction. They probably had been broken down into intermediate complexes as rapidly as they separated from the coarse particles of the straw. However, this was apparently not true for lignin. Analysis of the straw fraction showed that the lignin had decreased about as rapidly as the cellulose and pentosans, but when the sludge fraction was analyzed, it was noted that a large part of the lignin had not been decomposed but had accumulated in the sludge. In the mixture early loss of lignin was noted, followed by an apparent increase and finally by a decrease. No corresponding increase in lignin was observed in the straw fraction (fig. 5, *B*). Apparently most of the lignonitrogenous complexes were formed in the sludge fraction. This

accounts for the apparent increase in lignin (fig. 1). There was, however, a slight increase in Kjeldahl nitrogen in the lignin residue from the straw fraction (fig. 4).

After the third day, the percentage losses of pentosans and cellulose from the straw fraction were practically the same, indicating that a prefermentation period would be of little value in the manufacture of paper pulp from wheat straw. In breaking down the pentosans sufficiently, considerable cellulose would also be broken down. However, a short fermentation period might have value in making board pulp.

Little break-down of the constituents occurred in the seed (control) and in the straw flour and the chopped straw fermented in water (controls), as shown by the data in tables 2 and 7.

TABLE 7.— *Decomposition of constituents in wheat straw fermented in water at 50° to 55° C. for 20 days*

Constituents		Wheat-straw flour			Chopped wheat straw -		
		Before fermentation	After fermentation	Decomposed[1]	Before fermentation	After fermentation	Decomposed[1]
Total solids	grams	28 4	27 5	0 9	28 4	27 0	1 1
Volatile solids	do	26 4	25 6	8	27 0	25.9	1. 1
Pentosans	do	8 3	7 7	.6	8. 5	8 3	.2
Crude cellulose	do	15 5	15.0	5	15. 1	15 1	.0
Pentosans in the cellulose	do	5 2	4 9	.3	4 7	4 7	.0
Cellulose (corrected)	do	10 3	10 1	.2	10 4	10 4	0
Alpha cellulose	do	10 2	9 8	.4	9 8	10 1	+.3
Lignin	do	4 3	4 5	+ 2	4 4	4 4	0
Kjeldahl nitrogen in the lignin	percent	5	8		1 0	.2	

[1] + indicates a gain.
[2] Corrected for ash and pentosans.

The use of this anaerobic type of fermentation should be of value in composting straws. The rate of break-down of the constituents is much higher by this method than that reported in most of the literature on composting, and a gas is produced that could be used either for heating or for the production of power. However, no work has been done to determine the value of the residue as a fertilizer.

PRODUCTION OF GAS

The volumes of gas produced, corrected to 30 inches (760 mm.) of mercury and 60° F. (15.6° C.), are recorded in table 8 and shown graphically in figure 6. The total volume of gas produced per gram of volatile solids present appears to be appreciably lower than the corresponding values for many of the other agricultural wastes, especially cornstalks (3).

No significant differences were noted in the volumes of gas produced from the straw flour and from the chopped straw throughout the entire fermentation period. The size of the particles of the material seemed to have little effect on the rate of gas production, indicating that no appreciable time would be saved by grinding wheat straw. This behavior is different from that found in studies on cornstalk fermentation (4, 5), where the fine material, the flour, fermented and produced gas much more rapidly than the coarse material, the chopped cornstalks. Since wheat straw and cornstalks are similar chemically, containing approximately the same quantities of cellulose,

pentosans, and lignin, one might expect these two materials to react very much alike when fermented. They did not, however, and some factor or factors other than gross chemical composition probably influenced the fermentation.
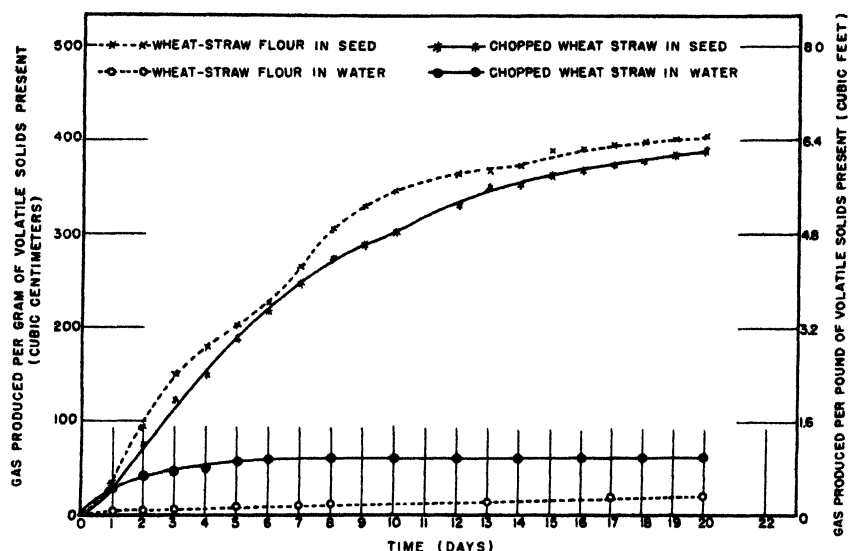

FIGURE 6.— Quantity of gas produced from wheat straw in an active methane-producing seed and in water at 50° to 55° C.

TABLE 8.— *Gas produced from the wheat-straw mixtures and seed control fermented for various periods at 50° to 55° C.*[1]

Time fermented (days)	Wheat-straw flour				Chopped straw				pH of seed control
	In active seed [3][4]		In tap water		In active seed [4][5]		In tap water		
	Gas produced	pH of mixture	Gas produced	pH of mixture	Gas produced	pH of mixture	Gas produced	pH of mixture	
	Cc.		Cc.		Cc.		Cc.		
1	32.8	6 8	3 2	6.0	25.2	6.8	27.2	6.3	7.2
2	94.5	6.8	4 8	6.0	75.4	6.8	40.7	6.1	7.6
3	150.6	7.0	6.4	6.0	122.8	6 9	47.6	6 1	7.7
4	178.2	6 9	6.4	6.0	153.7	6 9	51.1	6.1	8.0
5	202.7	7.0	9.5	6.0	187.4	6 9	58.0	6.3	7.7
6	224.7	7.0	9.5	6.0	217.6	7.0	61.2	6.1	8.0
7	264.6	7.0	11 1	6 0	246 3	6.9	61.2	6.1	8.2
8	305.5	7.2	12 7	6.3	273.0	6.9	64.6	6.3	8.2
9	326.5	7.0	12.7	6.1	286.8	6.9	64.6	6.1	8.1
10	344.9	7.1	12.7	6.1	302.2	6.9	64.6	6.3	8.1
12	359.0	7.2	12.7	6.1	331.2	7.0	64.6	6.4	8.1
13	375.7		15.9		351 3		64.6		
14	382 7	7.2	15.9	6.1	354.6	7.2	64.6	6.5	8.1
15	389.4		15.9		361.3		64.6		
16	392.8	7.3	15.9	6.3	367.8	7.3	64.6	6.3	8.2
17	396.0		19.0		374.5		64.6		
18	399.7		19.0		381.3		64.6		
19	401.4		19.0		388.1		64.6		
20	404.3	7.3	19 0	6.3	391.9	7.3	64.6	6.5	8.1

[1] Corrected to 30 inches of mercury and 60° F.
[2] Computations were made after corrections were made for gas produced in the seed control. The total gas produced by the seed control was only 45 cc. and this was produced on the first day.
[3] Average gas composition: CO_2, 29.4 percent; H_2, 2.4 percent; CH_4, 56.5 percent.
[4] Corrected for 45 cc. of gas produced by seed control.
[5] Average gas composition: CO_2, 27.6 percent; H_2, 2.5 percent; CH_4, 58.2 percent.

The quantities of gas (*4*) that would be available if gas were produced from all the cellulose and pentosans broken down are shown in table 9.   These calculated values and the actual quantities of gas produced are compared graphically in figure 7 for the straw flour
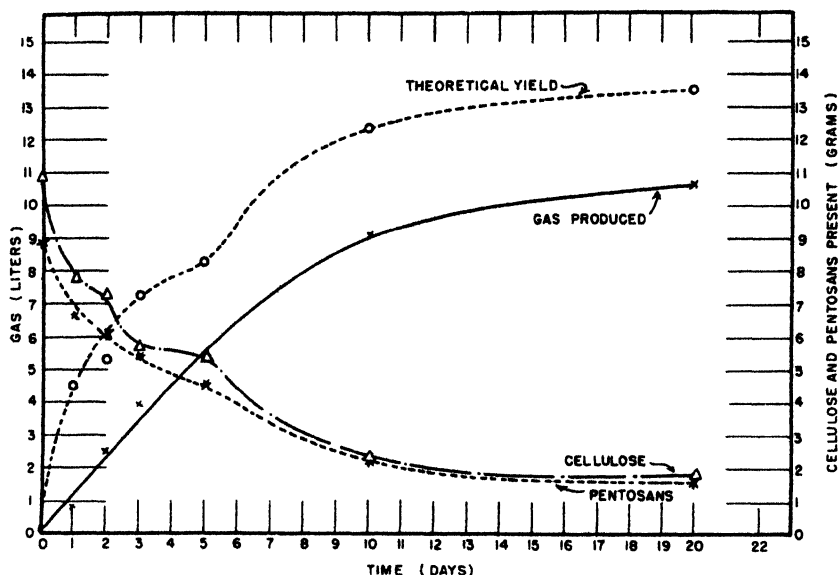
FIGURE 7.--Quantity of gas produced from 28.35 gm. of wheat-straw flour in an active methane-producing seed at 50° to 55° C., the theoretical yield based on the quantities of cellulose and pentosans decomposed, and the quantities of cellulose and pentosans left after the fermentation.
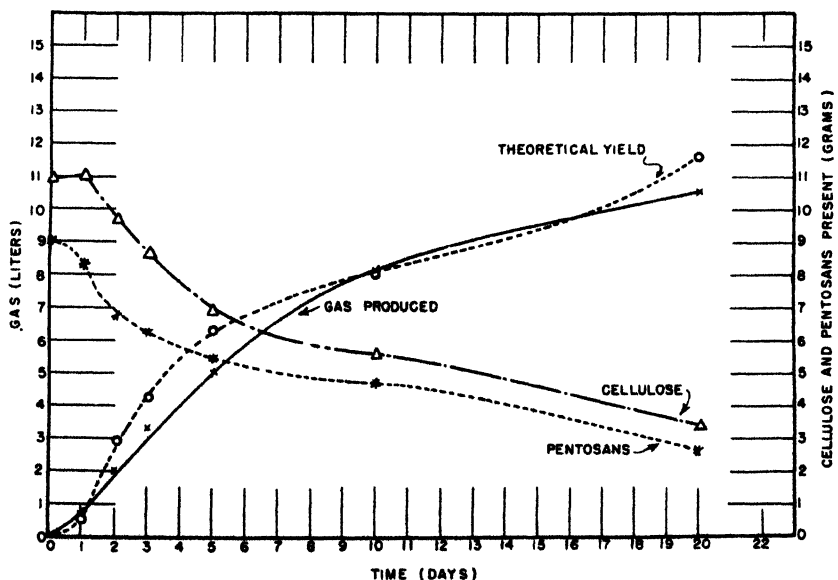
FIGURE 8.—Quantity of gas produced from 28.37 gm. of chopped wheat straw in an active methane-producing seed at 50° to 55° C., the theoretical yield based on the quantities of cellulose and pentosans decomposed, and the quantities of cellulose and pentosans left after the fermentation.

and in figure 8 for the chopped straw. The quantities of cellulose and of pentosans remaining are also shown in these figures.

In the straw-flour fermentation the pentosans and the cellulose broken down could have produced a quantity of gas considerably larger than that actually produced. As has already been noted, the total quantity of gas produced was considerably less than that produced from an equal quantity of cornstalk flour (4, 5). Inasmuch as these two waste materials are very similar chemically, it is unlikely that the variation in gas production by the two materials was due to a difference in gross chemical composition. The difference might be attributed to their physical structure or the configuration of their components, but this probably was not the sole reason, since the actual break-down of pentosans and of cellulose in the wheat-straw flour tends to approach the corresponding break-down in the cornstalk flour. Some intermediate reaction in the wheat-straw-flour fermentation may have exerted a depressing action on the production of gas by the complexes resulting from the cellulose and pentosan break-down. Further investigation is necessary to ascertain the cause of this behavior.

TABLE 9.—*Quantity of gas produced from wheat-straw flour and from chopped wheat straw and the theoretical yield based on the quantities of pentosans and cellulose decomposed after fermentation for various periods*

Time fermented (days)	Wheat-straw-flour mixture [1]					Chopped wheat-straw mixture [1]				
	Pentosans decomposed	Cellulose decomposed	Gas [2]		Ratio of theoretical yield to actual yield	Pentosans decomposed	Cellulose decomposed	Gas [2]		Ratio of theoretical yield to actual yield
			Actual yield	Theoretical yield based on quantity of pentosans and cellulose decomposed				Actual yield	Theoretical yield based on quantity of pentosans and cellulose decomposed	
	Gm.	*Gm.*	*Cc*	*Cc*		*Gm.*	*Gm.*	*Cc.*	*Cc*	
	2 2	3 1	865	4,445	5 1	0.6	+0 1	680	535	0 8
2	2.7	3 6	2,495	5,285	2 1	2 2	1 3	2,035	2,965	1 5
3	3 4	5 2	3,975	7,270	1 8	2 7	2 3	3,315	4,240	1 3
5	4 3	5 5	5,350	8,215	1 5	3 5	4 0	5,060	6,340	1.3
10	6 6	8 5	9,105	12,030	1 4	4 3	5 3	8,160	8,035	1 0
20	7 1	9.1	10,670	13,510	1 3	6 4	7.3	10,580	11,610	1 1

[1] 30 gm.
[2] Corrected to 30 inches of mercury and 60° F

In the chopped-straw fermentation practically all the cellulose and pentosans decomposed could be accounted for by the gas produced (fig. 8). This was in marked contrast to the findings in the straw-flour fermentations. As already pointed out, the total quantity of gas produced by the chopped straw was less than that produced by chopped cornstalks (4, 5). However, as all the cellulose and pentosans lost from the chopped straw could be accounted for by the gas produced, no lag period (time from decomposition to production of gas) occurred, as was the case with the chopped cornstalks. Probably no

depressing action, such as was noted in the straw-flour fermentation, was exerted on the production of gas by the intermediate complexes resulting from the break-down of the cellulose and pentosans. Very likely the principal reason for the difference in the quantities of gas produced by the chopped cornstalks and the chopped straw was a difference in the physical structure or the configuration of their components. The influence of physical structure would probably be more pronounced in the chopped material than in the flour.

SUMMARY AND CONCLUSIONS

Wheat-straw flour and chopped wheat straw were fermented for 20 days at 50° to 55° C. The pH was determined periodically. Daily measurements of the gas produced were made.

Percentage decomposition of cellulose and pentosans was high in the straw-flour fermentation. Little difference in the percentage decomposition of cellulose and of pentosans was noted, probably indicating that no selective fermentation took place. Lignin appeared to be the most resistant of these three major plant constituents, although appreciable break-down of lignin was observed during the later stages of the fermentation.

The decomposition of cellulose and of pentosans was much smaller in the chopped straw than in the straw flour. The break-down of pentosans and of cellulose was practically the same, and no selective fermentative action was noted. Only slight decomposition of lignin was noted throughout this fermentation.

In both fermentations, the formation of lignonitrogenous complexes was indicated. The actual break-down of lignin, therefore, was probably greater than that shown by the analysis. In the straw-flour fermentation, in which appreciable decomposition of lignin occurred during the latter part of the fermentation, a distinct decrease was noted in the Kjeldahl nitrogen content of the lignin residue after 20 days' fermentation, indicating that the lignonitrogenous complexes were being appreciably attacked.

The values for alpha cellulose corresponded closely to the values for the Norman and Jenkins' cellulose corrected for ash and pentosans. Apparently, these values may be used interchangeably for material of this type. This application would be of value in analyses in which an adequate quantity of sample for the actual alpha cellulose determination is not available.

Approximately the same volumes of gas were obtained from the straw flour (10,670 cc.) and the chopped straw (10,580 cc.). This was interesting in view of the fact that considerably greater quantities of the straw-flour constituents were broken down during the fermentation. In the straw-flour fermentation a depressing action probably was exerted on the production of gas by the complexes resulting from the break-down of the cellulose and the pentosans. If all the material broken down had produced gas, much greater quantities of gas would have been produced.

The quantity of gas produced per unit weight (gram of volatile solids) of raw material was considerably less for the wheat straw (flour, 404 cc.; chopped, 392 cc.) than was previously noted for cornstalks (flour, 543 cc.; chopped, 401 cc.).

Possibilities for the utilization of a fermentation such as is reported herein in the manufacture of paper pulp from wheat straw do not seem

promising. The loss of cellulose is too high and too nearly the same as the loss of pentosans to make this a promising paper-pulping procedure. However, the use of a short fermentation period may have some value in the making of board pulp.

This type of anaerobic fermentation may have some value in composting straws.

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EFFECT OF VARIOUS CONCENTRATIONS OF PAPAIN AND POTASSIUM IODATE ON THE LOAF VOLUME OF BREAD [1]

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INTRODUCTION

The mechanism of the action of chemical bread improvers has recently been the subject of wide discussion. Jørgensen (*9, 10, 11, 12, 13*),[2] and Balls and Hale (*2, 3*) have advanced the theory that the improvement of baking quality caused by oxidizing agents, such as potassium bromate and potassium iodate, is due to the fact that they inactivate the proteinases of flour, thereby inhibiting the action of these enzymes on the flour proteins. These writers also believe that the flour proteinases belong to the papain group.

This theory is supported by Flohil (*6*) and is opposed by Read and Haas (*16, 17, 18*), Carbonnelle (*5*), and Ritter (*19*). The arguments for and against the theory have been fully and ably summed up by the opposing sides in recent articles (*12, 13, 14, 18*).

In studies on the total and free amylase content of ungerminated seeds, baking tests were made on a sample of commercial wheat flour to which were added various concentrations of papain and potassium iodate. The results, reported here, may throw some added light on the disputed theories with regard to the mechanism of action of certain bread improvers.

PROCEDURE

The baking tests were conducted according to the standard procedure of the American Association of Cereal Chemists (*1*). The respective quantities of papain and potassium iodate specified in table 1 were added to the water (60 cc. per 100 gm. of flour) before it was mixed with the flour. The concentrations, percentage as well as molecular, are given on the basis of the water added. These tests were carried out in three series of bakings on different days. The respective concentrations of papain were the same in all three series. They ranged from 0.0005 to 0.01 percent, or from 0.3 to 6.0 mg. per 100 gm. of flour. Balls and Hale, who were the first to obtain a depression in the loaf volume of bread with papain, used 50 mg. per 315 gm. of flour or at the rate of 16 mg. per 100 gm. of flour. The concentrations of the potassium iodate solution, however, varied in the different series. They ranged from 0.00002 to 0.00008 molar or from 0.25 mg. to 1.0 mg. per 100 gm. of flour.

RESULTS

Table 1 and figures 1, 2, and 3 deal only with the loaf volume of the bread. The data in table 1 represent the products of the longitudinal and transverse circumferences of the loaves and serve only as a numerical expression of the relative volume of breads obtained with the vari-

ous concentrations of papain with and without potassium iodate. The measurement data and the photographs are in good agreement.

In the first series of bakings (fig. 1), the concentration of the potassium iodate solution was 0.00008 molar (1 mg. in 100 gm. of
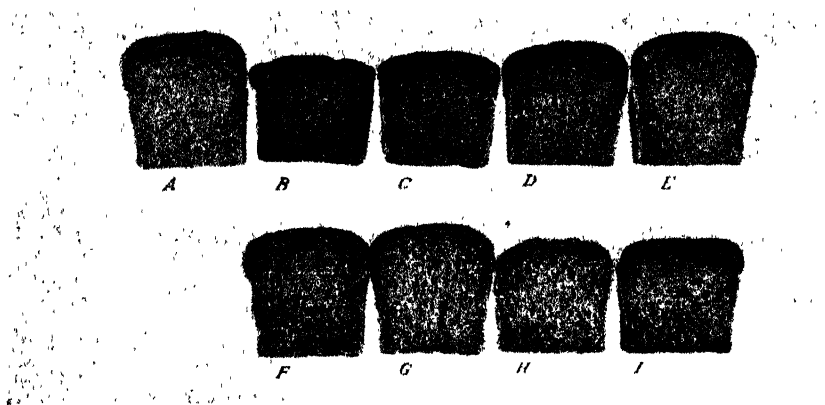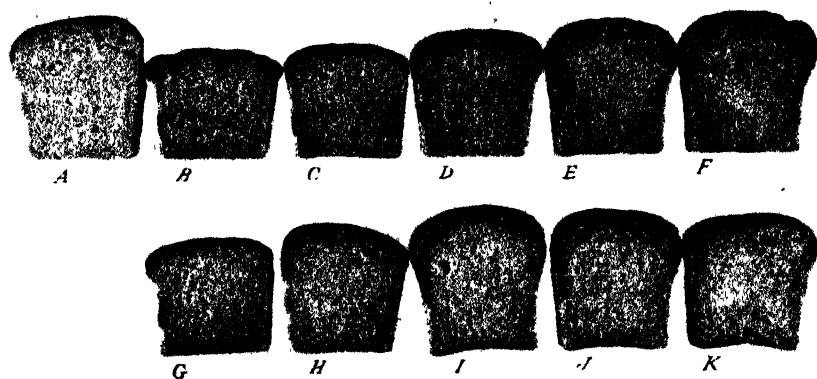

FIGURE 1.—Bread made with various concentrations of papain and potassium iodate. No papain or potassium iodate was added to *A* (control); to *B*, *C*, *D*, and *E*, papain was added in concentrations of 0.01, 0.005, 0.002, and 0.001 percent, respectively; to *F*, *G*, *H*, and *I*, an 0 00008 molar solution of potassium iodate (1 mg.) was added, together with 0.01, 0.005, 0.002, and 0.001 percent, respectively, of papain.

FIGURE 2.—Bread made with various concentrations of papain and potassium iodate. No papain or potassium iodate was added to *A* (control); to *B*, *C*, *D*, *E*, and *F*, papain was added in concentrations of 0.01, 0.005, 0.002, 0.001, and 0.0005 percent, respectively; to *G*, *H*, *I*, *J*, and *K*, a 0.00004 molar solution of potassium iodate (0.05 mg.) was added, together with 0.01, 0.005, 0.002, 0.001, and 0.0005 percent, respectively, of papain.

flour). Without potassium iodate the loaf volume consistently decreased with the increased concentration of papain. The greatest depressions, caused by the concentrations of 0.01 and 0.005 percent of papain (6 and 3 mg. per 100 gm. of flour, respectively) were overcome to a large extent by the potassium iodate. But with the concentrations of 0.002 and 0.001 percent of papain (1.2 and 0.6 mg.

respectively per 100 gm. of flour), the loaf volume was less with potassium iodate than that of the bread made without potassium iodate. This would indicate that these low concentrations of papain were not sufficient to overcome the depressing effect of an 0.00008 molar potassium iodate solution. That some concentrations of potassium iodate added singly depress the loaf volume of bread is shown by the work of Jørgensen (9) and by unpublished results of this laboratory.

The concentrations of potassium iodate that evidently caused a depression in the loaf volume of bread in these experiments are much lower than those that caused a depression in Jørgensen's tests. The

FIGURE 3. Bread made with various concentrations of papain and potassium iodate. No papain or potassium iodate was added to *A* (control); to *B*, *C*, *D*, and *E*, papain was added in concentrations of 0.01, 0.005, 0.002, and 0.0005 percent, respectively; to *F*, *G*, *H*, and *I*, a 0.00002 molar solution of potassium iodate (0.25 mg.) was added, together with 0.01, 0.005, 0.002, and 0.0005 percent, respectively, of papain.

most likely explanation of the discrepancy is that different flours were used in the two sets of experiments.

In the second series the concentration of the potassium iodate solution was 0.00004 molar (0.5 mg. in 100 gm. of flour) or half that of the first series. Here, too, the results show that the depression in loaf volume was directly related to the concentration of papain. It is further shown that the concentration of 0.00004 molar of iodate was not so efficient in counteracting the depressing effect of 0.01 and 0.005 percent of papain (6 and 3 mg. per 100 gm. of flour) as the concentration of iodate used in the first series but it completely overcame the depressing effect of 0.002 percent of papain. These results indicate that the concentrations of 0.001 and 0.0005 percent of papain (0.6 and 0.3 mg. per 100 gm. of flour) were not strong enough to counteract the depressing effect of this concentration of potassium iodate.

TABLE 1.—*Loaf volume* [1] *of bread obtained with various concentratons of papain and potassium iodate*

Concentration of papain solution (percent) [2]	Series 1		Series 2		Series 3	
	Without potassium iodate	With an 0.00008 molar solution of potassium iodate [3]	Without potassium iodate	With a 0.00004 molar solution of potassium iodate [4]	Without potassium iodate	With a 0.00002 molar solution of potassium iodate [5]
None	199		209		216	
.01	156	189	160	164	165	164
.005	162	188	162	181	177	180
.002	177	158	182	212	197	206
.001	192	159	196	203		
.0005			205	190	211	224

[1] Longitudinal circumference multiplied by transverse circumference. The figures are numerical expressions of the relative volume of the loaves.
[2] On the basis of water added. To obtain percentages on the basis of flour, multiply by 0.6.
[3] 1.0 mg. in 100 gm. of flour.
[4] 0.5 mg. in 100 gm. of flour.
[5] 0.25 mg. in 100 gm. of flour.

In the third series, in which a 0.00002 molar (0.25 mg. in 100 gm. of flour) potassium iodate solution was used, the results consistently follow the same trend as in the two previous series. This weakest concentration of potassium iodate used completely counteracted the depressing effect of only the weakest concentration of papain.

The results of these three series of baking tests indicate clearly that potassium iodate, which is classed among the bread improvers, counteracts the depressing effect of an added proteinase on the loaf volume of bread. Flohil (*6*) was able to overcome the depression in loaf volume of bread caused by papain by adding potassium bromate, another bread improver. Read and Haas (*17*) admit that potassium bromate inhibits the proteolytic action of papain but doubt whether the wheat proteinases belong to the papain group. The evidence in the literature indicates that at least some of the proteinases of wheat belong to the papain group (*2*, *4*).

The results also show that under certain conditions the proteinases in flour may be beneficial, because they tend to overcome the depressing effects of oxidizing agents, such as potassium iodate. Possibly flour contains natural oxidizing substances, an excess of which would depress the loaf volume just as an excess of potassium iodate does. Under such conditions the addition of a proteinase might prove beneficial. Indeed, Read and Haas (*16*) found that the addition of proteinases had a beneficial effect on the baking qualities of flours that develop "bucky doughs."

DISCUSSION

In the baking tests reported here the loaf volume was depressed by the added papain in direct relation to its concentration, the added potassium iodate counteracted this effect, and an excess of iodate in turn depressed the loaf volume. Since it is generally accepted that the loaf volume of bread is associated with the strength of the gluten, the logical interpretation of these findings would be that the papain attacked the proteins, destroying their strength, and that potassium

iodate counteracted the effect of the papain. Consistent with this reasoning is the theory that potassium iodate, added in excess, depressed the loaf volume of the bread because it carried the inhibition of the proteinases too far, as a certain degree of proteolytic action is supposed to be necessary for the proper maturing of the gluten. This is essentially the theory advanced by Jørgensen (*9, 13*) and by Balls and Hale (*2, 3*).

Is a different interpretation of these facts possible?

It is known that papain liberates amylase in some ungerminated cereals (*7*). The tests reported here showed that papain added to the flour in the concentrations used in the baking tests also liberates amy-

TABLE 2.—*Effect of various concentrations of papain on the development of diastatic power in flour*

Concentration of papain (percent)[1]	Diastatic power, in degrees Lintner, after—				
	3 hours	24 hours	48 hours	72 hours	144 hours
None	26. 5	25 6			
.5	120. 5	120. 5			
.01	111 1	117. 7			
.005	89. 3	117. 7	119. 0		
.0025	66 7	85. 5	109 0	115 0	120 5
.001	54 3	86. 2	89 3	104. 2	116 2
.0005	43 5	55. 6	59. 2	62. 2	72. 0

[1] On the basis of water added.

lase (table 2). Even the lowest concentrations of 0.0005 percent of papain had a pronounced effect in increasing the diastatic power of the flour. Potassium iodate in the concentration that counteracted the effect of papain and depressed the loaf volume of the bread in the baking tests (table 1) also depressed the diastatic activity of the flour (table 3). The extractions of the amylase in the last two experiments (tables 2 and 3) were carried out at about 70° F., according to the approximate procedure of the British Institute of Brewing (*8*). The effect of papain on the liberation of the flour amylase and the hydrolizing power of the latter, as well as the inhibiting effect of the potassium iodate, would be expected to be stronger at the temperature of dough ripening and of the first stages of baking (before the bread reaches the temperature of inactivation of the enzymes), than at 70° F. Therefore, while it is realized that the diastatic power of flour as determined by the Lintner method is not the only factor that conditions diastasis in dough, is it not still possible that the respective effects of the papain and potassium iodate on the loaf volume in these baking tests were due to their effect on the liberation or depression of the flour amylase, largely beta amylase? Indeed, Stamberg and Bailey (*20*) found that added beta amylase produced poorer loaves of bread than the controls. Kosmin (*15*) also found that excessive starch hydrolysis is injurious to the baking qualities of flour but attributed this effect to the alpha amylase. This is at variance with the results of Stamberg and Bailey, who found that the baking qualities of bread were improved by the addition of alpha amylase (*20*). Kosmin based her conclusions on results obtained with sprouted-wheat flour, while Stamberg and Bailey used separate alpha and beta amylase preparations.

TABLE 3.— *Effect of various concentrations of potassium iodate on the development of diastatic power in flour*

Concentration of potassium iodate [1]		Diastatic power, in degrees Lintner, after—				
Molar	Percentage	3 hours	24 hours	48 hours	72 hours	144 hours
None	None	23.2	20.8	19.2	19 2	
.005	.11	11.3	3 0	3 0		
.00008	.0017	23 1	17.6	13 5	11 3	9 8
.00004	.00085	22.7	18 9	16.3	15.1	12 1
.00002	.00042	23.2	20 0	19 5	17 1	11 3

[1] On the basis of the water added.

The beneficial effect of natural aging of flour on the baking qualities of bread, which Balls and Hale (*2, 3*) attribute to its inhibitive effect on the flour proteinases, may similarly be explained by its effect on the diastatic power of the flour, for the results presented here (controls in tables 2, 3, and 4) indicate that the diastatic power of flour gradually declines with time. The changes involved are relatively small, but they have been consistently verified in many tests.

In the concentration commonly used in bread making, however, sodium chloride also has a strong stimulating effect on the diastatic activity of flour (table 4). Even one-twentieth of the salt concentra-

TABLE 4.— *Effect of various concentrations of sodium chloride on the development of diastatic power in flour*

Concentration of sodium chloride (percent)[1]	Diastatic power, in degrees Lintner, after			Concentration of sodium chloride (percent)[1]	Diastatic power, in degrees Lintner, after—		
	3 hours	24 hours	48 hours		3 hours	24 hours	48 hours
None	33 3	29 4	26 7	.5	96 2	94 0	93 5
2.0	99 0	104 2	103 1	.25	86 2	88 5	87 7
1.0	96.2	100 0	101 1	.1	70 4	70 4	70 9

[1] On the basis of the water added.

tion used in the baking test causes a distinct stimulation of the diastatic power of flour. The maximum stimulation caused by salt, however, does not reach that caused by papain, and there are other differences between the action of papain and that of salt, which will be discussed elsewhere. The 2 percent of sodium chloride used in these baking tests did not cause any depression in loaf volume, notwithstanding the fact that it stimulated the diastatic activity of the flour. This would seem to exclude the possibility of explaining the results of the baking tests reported here by the effects of papain and potassium iodate on the stimulation or inhibition of the diastatic power of flour.

SUMMARY

The results of baking tests show that there is a consistent interaction between various concentrations of papain and potassium iodate in their effect on the loaf volume of bread.

When added singly, some concentrations of potassium iodate and papain depress the loaf volume of bread, the extent of depression being directly related to the concentration. When added together, certain of these concentrations counteract each other.

While papain stimulates diastatic activity and potassium iodate depresses it, the action of these substances on the loaf volume of bread cannot be ascribed to their effect on the diastatic power of the flour since sodium chloride, which is always used in bread baking, also stimulates diastatic activity, yet causes no depression in loaf volume.

The results seem to support the theory of the mechanism of action of bread improvers advanced by Jørgensen and Balls and Hale.

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INHERITANCE OF FACTORS INFLUENCING SUCROSE PERCENTAGE IN BETA VULGARIS [1]

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INTRODUCTION

A more comprehensive knowledge of the manner in which factors affecting sucrose percentage are inherited in sugar beets (*Beta vulgaris* L.) should be of considerable aid to sugar-beet breeders. Although use has been made of the sucrose percentage of individual plants in selection work, there is little in the literature that throws light upon the nature of inheritance of the ability of sugar beets to produce sucrose. Accordingly the investigation reported in this paper was made with the object of determining how sucrose percentage is affected by genetic factors in crosses between inbred lines of sugar beets differing in respect to their mean sucrose percentage.

REVIEW OF LITERATURE

Bougy (*1*) [3] stated that in crosses of sugar beets with mangel-wurzels the sucrose percentage of the F_1 was higher when mangel-wurzels were used as the female parent than when sugar beets were so used. However, the results were found to depend to some extent upon the variety of mangel-wurzel used as a parent in the cross. His conclusion was based on limited data. In the segregating generations Bougy found that the plants were intermediate between the parents and that ordinarily they were closer to the parent higher in sucrose percentage. Certain roots surpassed the higher parent, but only very rarely was the sugar content of the lower parent recovered.

In later investigations, the F_1 progenies of reciprocal crosses between the Kuhn sugar beet × mangel-wurzel and mangel-wurzel × Kuhn sugar beet reported by Colin and Bougy (*2*) averaged, respectively, 11.2 and 7.6 percent of sucrose. These results were based on the average of 20 plants of the first cross and 18 plants of the second cross. At another location, Kuhn × mangel-wurzel and mangel-wurzel ×

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Kuhn F_1 crosses averaged 12.60 and 12.58 percent sucrose, respectively, in 1936, and 10.76 and 10.96 percent, respectively, in 1937. In 1937, at the same location 5 plants of the cross Vilmorin A sugar beet × mangel-wurzel and three plants of mangel-wurzel × Vilmorin A averaged 14.05 and 12.00 percent, respectively.

In the same paper Colin and Bougy reported four tests made in 1928 to 1931 in which crosses of sugar beets × mangel-wurzels and their reciprocals averaged 15.99 and 16.56 percent sucrose, respectively. The number of F_1 plants in each cross was not reported. The arithmetic average of the parental lines in these f ur tests was 12.27 percent, which was much below the average of the F_1 plants.

The same authors reported that in a total population of 76 F_2 plants no root was as high in sugar percentage as the highest of the sugar-beet roots, but that 5 of the hybrids exceeded the poorest sugar-beet root. No F_2 roots were as low in sugar percentage as the lowest mangel-wurzel.

Savitsky (*6*), in a test of 62 Russian varieties of sugar beets, stated that the variability of sugar percentage was small compared to the variation of all other characters. The average sucrose percentage in the roots of the varieties tested ranged from 17.01 ± 0.09 to 14.47 ± 0.20.

Hybrids produced by the "method of alternate planting of stecklings of the varieties crossed" were studied by Savitsky (*7*). Crosses of high × low or low × high sugar varieties produced in general F_1 progenies with a sucrose percentage near the arithmetic mean of the two parent varieties. In crosses of this type, the sucrose percentage of the higher parent was rarely obtained in the F_1.

Savitsky (*8*) studied the species *Beta vulgaris* L. and found that the sugar beet was highest in sucrose percentage, followed in order by white mangold,[4] semisugar beet, table beet, and mangel-wurzel. He believed that as a result of long-continued selection the sugar beet differs from primitive forms, not only in high sucrose content but also in its ability to retain this high sucrose content under adverse environmental conditions. F_1 hybrids between white mangolds and sugar beets were found to be higher in sucrose percentage than the mean of the parents, and frequently approached the sucrose percentage of the sugar beet. Backcrosses of the F_1 hybrid with sugar beets produced progenies with a sucrose percentage nearly equal to that of the sugar-beet parent. The high sucrose percentage of the sugar beet × mangold hybrids persisted in F_2 and failed to segregate as expected, there being a preponderance of individual F_2 roots of high sucrose percentage.

From his observations, Savitsky concluded that the mangold possessed certain basic genes influencing sucrose percentage which closely resembled those of the sugar beet, and that the mangold lacked certain modifying factors necessary for high sucrose percentage which have been retained in the sugar beet through selection. The genetic constitution of the mangold was tested by means of crosses with mangel-wurzels compared with crosses of sugar beets with mangel-wurzels. It was found that the F_1 cross of mangold with mangel-wurzel produced nearly the same sucrose percentage as the F_1 cross of sugar beets with mangel-wurzels. The slight difference in the two crosses was attributed to the presence of modifying factors contained in the sugar beet.

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[4] The term "mangold" is normally used synonymously with "mangel-wurzel." As explained to Dr. G. H. Coons (via interpreter), and from the context of the paper, Dr. Coons believes that Savitsky refers to chard or chardlike plants.

In conclusion, Savitsky stated that a knowledge of beet genetics made it possible to synthesize a beet variety of high sucrose percentage in a few generations with only a limited number of plants.

As a result of correlation studies, Pack (4) found that the sucrose percentage of beets was positively and significantly correlated with such root characters as increased density, dry substance, extension of the shoulder beyond the first leaf scar, hardness of tissue, length, purity, refractometer reading, and roughness of skin. Top characters found to be associated with high sucrose percentage were darkness of leaf color, flatness of crown, and petioles of small cross-section area.

MATERIALS AND METHODS

The best inbred lines obtainable were used as parents in this study. Special emphasis was placed on as long a record of inbreeding as possible, but it was found that lines inbred for a period of years were not numerous. Two of the lines finally selected had a record of 5 or 6 years of inbreeding, and one line had been inbred for a shorter period but had originated from material that appeared homozygous in agronomic test plots.

It is generally recognized that sucrose percentage in the beet is influenced by environment as well as by genetic factors. For this reason it has been difficult to determine whether or not a line was homozygous until progeny tests have been made by inbreeding a number of plants of the line and determining the variations within and between the selfed progenies.

Seed and roots were obtained from two inbred strains,[5] one of which, called line 1 in this paper, had a pedigree starting in 1915 as follows: *s-s-s-s-gr-s-gr-gr-gr;* the other, called line 2 in this paper, had the pedigree *s-s-s-s-s-s-gr-gr.* In the production of these strains, space isolation (*s*) was relied upon to obtain selfing. The groupings (*gr*) also were made at isolated locations by combining the progeny of several roots. A detailed discussion of these lines, under the designations strain 1 and strain 9a, has been given by Stewart, Lavis, and Coons (9).

Roots were obtained from one additional inbred line, 4276,[6] which was reported to be highly self-fertile but very low in sucrose percentage. The line had been inbred for two generations. Each of the three lines selected possessed distinct morphological characters and could be easily distinguished when grown in the field.

Bulk seed [7] of lines 1 and 2 was used, i. e., seed from isolated group increases, as well as seed of the F_1, F_2, and first backcross generations. One lot of seed of a commercial brand, which could be classed as of intermediate type with respect to sucrose percentage and root yield, was used as a check throughout these tests.

The inbred lines selected, particularly lines 1 and 2, were believed to be relatively pure. The crossing program was begun before the lines themselves could be thoroughly tested.

Six roots of each of the three lines were transplanted in the greenhouse during the winter of 1936–37. All three possible crosses between

these lines were made by means of controlled pollination. Sturdy branches with 8 to 12 well-placed flower clusters were selected for emasculation. It was observed that the stamens were more easily removed from the larger florets and that a higher percentage set seed than when smaller and more slowly growing florets were used. The tip of the branch with the youngest florets was removed, and leaflets along the branch were removed also. When there was little difference in the size of the lateral flowers as compared with the central flowers, the entire group was emasculated; otherwise, the lateral flowers were removed. Usually less mechanical injury resulted from emasculation than from removing the entire lateral flower, and for that reason many of the lateral flowers were emasculated and later pollinated.

Branches with emasculated flowers were kept covered with light paper bags to exclude foreign pollen. The stigma lobes were usually well opened 4 or 5 days after emasculation, and pollination at that time proved most effective.

For the production of selfed seed, unemasculated branches on each plant were covered with bags before the flowers had opened. The amount of selfed seed produced on these original plants was not large enough to permit of planting in the field in 1937.

Seed of each of the three crosses was planted in soil in paper tubes $2 \times 2 \times 7$ inches in greenhouse flats early in the spring. These plants were transplanted to the field at Waseca, Minn., on May 22, 1937. The paper was removed from the core of soil and the core then planted in a hole of suitable size. Thus, minimum injury resulted to the roots of the young plants, and a high percentage of the transplants survived. The normal growth of the taproot was not seriously affected by this method of transplanting, and a higher percentage of the seedlings was saved than would have been the case had the seed balls been planted directly in the field.

The selfed seed of each line was planted in pots in the greenhouse, and the plants transferred to the field. In the fall they were removed to the greenhouse and again self-pollinated to continue the inbred lines.

Two roots of good size and shape were selected from the F_1 population of each cross, and after a short period of storage at a temperature of 34° F. they were replanted in the greenhouse.

Backcrossed seed was obtained during the winter of 1937–38, by means of controlled pollination, by the method used in making the original crosses, as described above. All three of the F_1 crosses, 1×2, 1×4276, and 2×4276, were backcrossed to the selfed plants of each of the parental lines of the original cross.

In the fall of 1938 a random sample of roots was selected from each of the parental lines, F_2 populations, and backcross populations. In addition, the highest and lowest testing individuals of each population were selected for further study. Since further study of the parental lines to determine the variability within each inbred line seemed desirable, selfed progenies of individual plants from the random lot selected from each inbred line were used to determine the variability in sucrose percentage existing within the lines. The random lots selected from within the segregating populations were used to show how rapidly types with various sucrose percentages could be fixed. Those roots with sucrose percentages at the extremes of the frequency

distributions were of particular interest, since it was possible that in the F_2 population individual roots relatively homozygous for sucrose percentage might have occurred.

The roots thus selected were planted in 8-inch pots in the greenhouse and bagged for selfing as the first flowers opened. Out of a total of 350 roots planted, 300 plants set seed, although in some instances the amount was small and in others the germination was low. Selfed seed of the 300 families was planted in the field in 1939.

Because of the small amount of seed, it was impossible to plant at customary rates and follow by thinning to one plant in each place. Rather it seemed advisable to make the smaller lots of seed go as far as possible by planting single seed balls and thus obtaining the maximum number of plants.

In 1937 the spacing employed was 36 by 30 inches. From a study of the effect of missing plants upon the weight of roots adjacent to a skip in 18 by 18 inch planting in commercial fields, it was found that the very wide spacings used in 1937 were probably unnecessary, hence a 20 by 20 inch spacing was used in 1938 and 1939.

As previously mentioned, the F_1 plants were started in the greenhouse and transplanted in the field in 1937. Since larger quantities of seed were available for the 1938 and 1939 plantings, they were made directly in the field, dropping one seed ball, or more, if the quantity available permitted, in each hill spaced 20 by 20 inches. The stands were thinned, at the six-leaf stage, to a single plant in each place.

Since the original lots of F_1 seed produced by controlled pollination consisted of 25 to 100 seed balls each, each cross was planted in a single row of from 25 to 52 plants, without replication. Every alternate row in the test plot was planted to the check variety to serve as a measure of the variability of the soil in the test plot.

In 1938, three replications of single-row plots 125 feet in length were used. One row of the check variety was employed in each replication, and since the results showed that there was little variation from one replication to the next, the data for the individuals of each population were brought together from all three replications without correction for block effect.

In 1939 a much larger plot was used. The 300 families were planted in two replications of single-row plots 41⅔ feet in length. The check variety was planted in every sixth row. Ten-beet composite samples were taken from each check plot, and the sucrose percentage was determined. As in previous years, the performance of the check with respect to sucrose percentage in the beet was strikingly uniform over the entire test plot, and it was unnecessary to make any correction for sucrose percentage between the two replications.

Table 1 summarizes the various populations that were grown in the field in each of the 3 years.

The individual roots were tested for sucrose percentage by the Sachs-Le Docte cold-water digestion method.

Environmental conditions favored normal growth each year. The check variety yielded 9.3, 17.6, and 17.0 tons per acre, with a mean sucrose content of 13.62, 13.07, and 10.92 percent for the years 1937, 1938, and 1939, respectively. Yield per acre was lower in 1937 than in 1938 or 1939, due in part to the wider spacing used that year. Sucrose percentage was reduced somewhat in the check, inbred lines,

and crosses because of the wide spacing used. Sucrose percentage was generally low in all fields in southern Minnesota in 1938.

TABLE 1.— *Lines,[1] crosses, and backcrosses used in study of inheritance of sucrose-percentage factors, 1937–39*

1937[2] F_1	1938			1939		
	P_2	F_2	Backcrosses	P_3	F_2	Backcrosses selfed
1 X 2	1	1 X 2	(1 X 2) X 1	1	1 X 2	(1 X 2) X 1.
1 X 4276	2	1 X 4276	(1 X 2) X 2	2	1 X 4276	(1 X 2) X 2
2 X 4276	4276	2 X 4276	(1 X 4276) X 1	4276	2 X 4276	(1 X 4276) X 1.
Check	Check		(1 X 4276) X 4276	Check		(1 X 4276) X 4276.
			(2 X 4276) X 2			(2 X 4276) X 2
			(2 X 4276) X 4276			(2 X 4276) X 4276

[1] The symbols P_1, P_2, and P_3 indicate the first, second, and third generations of inbreeding, respectively, after the original roots were received.
[2] A very limited amount of P_1 seed was produced in 1937, and the P_1 plants were grown in a special nursery.

EXPERIMENTAL RESULTS

VARIABILITY OF PARENTAL LINES

Before discussing the breeding behavior of the F_1, F_2, backcrosses, and F_3 lines, an analysis of the breeding behavior, after further selfing, of the three parental lines used in this study will be given. Lines 1 and 2 had a previous record of at least five generations of inbreeding, while line 4276 had been inbred for only 2 years. These lines were inbred for another 3 years, with paper bags used as a means of excluding foreign pollen.

Twenty roots of the parental lines grown in 1938 were selected from lines 1, 2, and 4276. These were selfed in the greenhouse during the winter of 1938–39, and progenies of all roots that produced a sufficient quantity of seed were grown in the field in 1939. The data obtained from these progenies are shown in table 2.

The P_3 families within line 1 varied widely in mean sucrose percentage and in uniformity. Culture 1 was highest in mean sucrose percentage and the most uniform, while culture 14 was lowest in sucrose and the most variable. The difference (3.53 ± 0.45) between the mean sucrose percentage of these two cultures was highly significant, and the difference between the standard deviations was found to be significant by using Fisher's (3) test of significance of the difference between two variances. The parents of these two progenies had been selected in 1938 as high and low, respectively, in sucrose percentage. The mean sucrose percentages and standard deviations of the other cultures were distributed between the extreme limits set by cultures 1 and 14. Cultures 1 and 9 appeared to be relatively homozygous, since most of the sucrose percentages of the individual roots fell into two classes in each case. Cultures 2, 4, 8, 12, and 14 seemed relatively heterozygous. It appeared, therefore, that line 1 was not as homozygous as might have been expected from its history of inbreeding prior to the time it was obtained for use in this study.

The P_3 progenies were much more uniform in line 2 than in line 1. The difference in mean sucrose percentage between culture 17 and culture 22, the highest and lowest testing cultures, was 2.09 ± 0.27 percent.

The parent roots of these two progenies were higher and lower, respectively, in sucrose percentage, than the average. All the other cultures had similar mean sucrose percentages. The standard deviations of the different P_3 progenies of line 2 were relatively similar. These facts indicated that, while line 2 was not completely homozygous, it was much more uniform than line 1.

TABLE 2.—*Frequency distributions* [1] *and means of sucrose percentages of P_3 progenies of roots selected from parental lines*

Line and 1939 culture No.	Sucrose percentage of P_2 parent	Roots in sucrose-percentage class—											Total roots	Mean sucrose percentage of P_3 progeny	Standard deviation
		4	5	6	7	8	9	10	11	12	13	14			
		No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.		
Line 1															
1	14 1						2	9	7	1			19	10 38±0. 14	0 63±0 10
2	13.7	1			2	3	9	2	1	1			19	8 73± .41	1.80± .29
3	13 3		1			9	7	6	1	1			25	8 96± 23	1 15± .16
4	13.2			1	2	2	5	7				1	18	9 03± .41	1 74± 29
5	13 2			1		1	7	12	3				24	9.58± 23	1 13± .16
6	13.0						3	7	5	2			17	10 29± .21	87± 15
7	12 5				1		5	7	3				16	9 58± 24	.94± 17
8	12 5	1	3		2	4	7						17	8 52± 42	1.71± .29
9	12 4					1	5	6	1				13	9.43± .20	.73± 14
10	12 1				1	3	8	3					15	8 83± 21	79± .14
11	11 4				1	1	6	5	2				15	9 40± 27	1.03± 19
12	11 4	1		2	1	5	11	6					26	8 45± 29	1 47± 20
13	10 8					6	9	3		1			19	8 94± 22	95± .15
14	10.7		4	6	6	1	2					1	20	6.85± 43	1.93± 31
Line 2															
15	15 2							2	4	1			7	10 70± .29	.76± .20
16	14 8							6	13	4	1		24	10 93± 14	71± .10
17	14 8							2	5	4	1		12	11 37± 21	72± .15
18	14 0						1	11	6				18	10.28± .11	46± 08
19	13 9							2	16	2			20	10 90± 09	.40± 06
20	13.8							7	6				13	10 39± 09	.33± 06
21	13 8							3	15	5			23	10 96± 13	.62± 09
22	9 8					3	7	7	1				18	9 28± 17	.73± 12
23	6 9							2	10	1			13	10 89± 17	.60± 12
Line 4276															
24	11 2		2	3	4								9	6 20± 20	61± 11
25	10 7	3	2										5	4 80± 19	42± 13
26	10.1				2								2	6.70± 00	00± 00
27	9 0		3	2									5	5 46± 12	27± .09
28	8 9			2									2	5.70± 00	00± 00
29	8 2				1	6	3	8	1				19	8 91± 24	1 03± .17
30	7 5	1	1			1							3	5 77± 93	1 61± .66
31	7 0			2									2	5 95± 26	.36± 18

[1] The frequency distributions in this and following tables were originally divided into 0.5-percent sucrose classes, from which means and standard deviations were calculated.

The number of plants in the P_3 progenies of line 4276 was small, with the exception of culture 29. Considerable difficulty was experienced in maintaining line 4276 from inbred seed produced under bags in the greenhouse, although the line was originally reported to be highly self-fertile. That culture 29 was more self-fertile and higher in mean sucrose percentage than the other cultures of line 4276 may have been the result of accidental cross-pollination in the previous generation, but it appeared similar morphologically to other cultures of the same line and its peculiar behavior was not suspected until the sucrose analyses were made. With the exception of culture 29, line 4276 appeared to be relatively homozygous for a low percentage of sucrose. The lack of correlation between the sucrose percentage of the P_2 parents and the mean sucrose percentages of their P_3 progenies ($r = -0.22$) indicated also that this line was relatively homozygous.

Line 4276 appeared about as uniform as line 2, if culture 29 is disregarded. This lack of uniformity of the parental lines made it necessary to interpret carefully the data obtained in F_1 and segregating generations of crosses 1×2 and 1×4276. The data obtained from cross 2×4276 have been considered the most reliable.

Correlations between the sucrose percentage of the P_2 mother beets and the mean of their P_3 progenies were calculated and found to be 0.59, 0.35, and −0.22 for lines 1, 2, and 4276, respectively. Only the first of these correlation coefficients was statistically significant, since the values of r required for odds of 19 : 1 were 0.53, 0.67, and 0.71, respectively. Although the number of comparisons for each line was small and the value of r required for significance was high in each case, the fact that there was a smaller association between the sucrose percentage of the P_2 mother beets and the mean of their P_3 progenies in lines 2 and 4276 than in line 1 furnished additional evidence that lines 2 and 4276 were more uniform than line 1.

TEST OF BULK SEED LOTS

Bulk seed of the parental lines 1 and 2, reciprocal F_1 crosses, the F_2 generation, and two backcrosses, together with the check variety, was planted in 1937 in single-row plots 132 feet long.[8] The data obtained from this test are given in table 3.

TABLE 3.—*Frequency distributions and means of sucrose percentages of parental lines, F_1 and F_2 crosses, and backcrosses grown from bulk seed in 1937*

| Line or cross | Roots in sucrose-percentage class— | | | | | | | | | | Total roots | Mean sucrose percentage | Standard deviation |
	8	9	10	11	12	13	14	15	16	17			
	No	*No.*	*No*	*No*	*No*	*No*	*No*	*No*	*No.*	*No*	*No.*		
1					2	26	8	4			40	13.28±0 11	0 68±0 08
2				8	15	5	5		1		34	12.30± 20	1 15± 14
1×2, F_1					13	14	4				31	12 55± .14	79± .10
2×1, F_1	1			1	8	21	9				40	12 75± .17	1 08± 12
1×2, F_2			3	3	11	14	8	2			41	12 65± .19	1.22± .13
1×(1×2)				1	11	16	9	4			41	13 09± .15	97± .11
(1×2)×1				3	12	16	7	3			41	12 71± .16	1 01± .11
Check			1	1	9	9	12	11	1	2	46	13 62± .21	1. 42± .15

The difference in mean sucrose percentage of the two parental lines was not as large as expected, since Stewart et al. (9) had reported a difference of 2.95 percent in 1932. Parental line 1 was significantly more uniform than the check variety, while line 2 was not significantly less variable than the check. The difference between the standard deviations of the reciprocal F_1 crosses approached significance. Both F_1 crosses were numerically less variable than the check, but the difference in variability between the F_1 cross and the check was significant only when line 1 was employed as the female parent. The F_2 cross was not significantly less variable than the check, while the two backcrosses were similar in variability, both being significantly less variable than the check variety. The relative uniformity of lines 1 and 2, as grown from bulk seed, did not agree with that obtained on further selfing and reported in table 2.

The parental lines differed significantly in mean sucrose percentage, but did not agree with the classification obtained by further selfing as shown in table 2.   Line 1, grown from bulk seed in 1937, was 0.98 ± 0.23 higher in mean sucrose percentage than line 2.   When grown in 1938 from seed produced by two further generations of inbreeding, line 2 was 1.28 ± 0.09 higher in mean sucrose percentage than line 1 (see table 5).   Presumably the genotype of the plants used as parents in this cross was not the same as the predominant type isolated by further inbreeding.

RECIPROCAL CROSSES

Reciprocal crosses between identical plants were made in order to check previous reports that reciprocal F_1 crosses may differ in sucrose percentage.   These reciprocal crosses were planted in such a way that the results from paired F_1 reciprocals could be studied in a critical manner.   The results are given in table 4.

TABLE 4.- *Sucrose percentage of reciprocal F_1 crosses between identical plants*

Cross	Roots	Sucrose	Difference
	Number	*Percent*	
1×2	15	13 62	....
2×1	15	13 57	0 05

The difference between the mean sucrose percentage of the paired F_1 progenies of reciprocal crosses was not of sufficient magnitude to be statistically significant.   Although the number of paired comparisons was small, it seems evident that when identical plants were crossed, the sucrose content of the progeny was not influenced by the direction in which the cross was made.

Since it seemed clear from this test that there was no difference between reciprocal crosses, no importance has been attached in this study to the direction in which the crosses were made.   In this paper, a cross reported as 1×2, for example, may contain the reciprocal cross, 2×1, as well.

F_1 AND F_2 CROSSES MADE BY CONTROLLED POLLINATION UNDER BAGS IN THE GREENHOUSE

The three F_1 crosses produced by controlled pollination under bags in the greenhouse were grown in 1937 in single-row plots.   The plants were spaced 36 by 30 inches, and no correction was made for missing plants, since the wide spacing reduced competition to such an extent that sucrose percentage would be affected but little.   In 1938 sufficient seed of the parental lines was available for planting, and the parental lines were grown together with the F_2 crosses in single-row plots, replicated three times.   The beets were spaced 20×20 inches in 1938, and skips in the stand were disregarded.   The frequency distributions, means, and standard deviations for the parental lines, F_1 and F_2 crosses, and the check variety are given in table 5.

TABLE 5.—*Frequency distributions and means of sucrose percentages of parental lines and F₁ and F₂ crosses from seed produced by controlled pollination*

Line or cross	Year grown	Roots in sucrose-percentage class—												Total roots	Mean sucrose percentage	Standard deviation
		7	8	9	10	11	12	13	14	15	16	17	18			
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*		
1×2, F₁	1937						2	25	13	7				47	13.48±0.11	0.76±0.08
1×4276, F₁	1937						2	12	5					19	13.09±.16	.69±.11
2×4276, F₁	1937				8	9	10	5						32	11.32±.18	1.02±.13
Check	1937				1	1	9	9	12	11	1	2		46	13.62±.21	1.42±.15
1	1938					20	73	52	4					149	12.24±.06	.71±.04
2	1938				1	2	6	47	70	11				137	13.52±07	.79±.05
4276	1938	12	62	56	11	3	1							145	8.51±.07	86±.05
1×2, F₂	1938				8	52	103	124	53	8				348	12.49±06	1 03±.04
1×4276, F₂	1938		3	7	17	43	65	30	7	2				174	11.59±.10	1.33±.07
2×4276, F₂	1938			5	40	51	27	11	1					135	10 93±09	1 05±.06
Check	1938				2	6	38	59	47	14	3			169	13.07±.08	1.07±.06

Since it was impossible to produce sufficient seed by controlled pollination to permit growing the parental lines and F₁ and F₂ crosses the same year comparison had to be made of populations grown in 2 successive years. The same lot of check seed was used in both years and served as a measure of the relative sucrose percentage and variability in the 2 years.

The three parental lines were less variable in sucrose percentage than the check variety, and the variability was not significantly different. On further selfing, line 1 proved to be more variable than lines 2 and 4276, as shown previously.

The average sucrose percentage of the F₁ crosses grown in 1937 was 12.63. The average of the parents grown in 1938 was 11.42 percent, which was lower than the average of the F₁ crosses by 1.21 percent. The check, grown in both years, was lower in sucrose percentage in 1938 by 0.55 percent. It appears, therefore, that the F₁ crosses were in general slightly higher in sucrose percentage than the average of the parents. The average of the F₂ crosses was 11.67 percent sucrose, which is only slightly higher than the average of 11.42 for the parents grown in the same year.

The F₁ generations of crosses 1 × 2, and 1 × 4276 were no more variable than their parental lines, while the F₁ of cross 2 × 4276 was more variable than either parent. The F₂ populations of crosses 1 × 2, 1 × 4276, and 2 × 4276 were more variable than the F₁.

The variability, as expressed by the standard deviation of the F₂ populations, was highest in cross 1 × 4276. In cross 2 × 4276 the standard deviation of the F₂ population was relatively low, in spite of the fact that the mean difference in sucrose percentage between the parent lines was 5.01 percent and the maximum amount of segregation would be expected to occur in F₂. In this cross the segregation in F₂ reached neither the upper limit of the parent higher in sucrose percentage nor the lower limit of the parent lower in sucrose percentage.

CROSS 1 × 2

The frequency distributions and mean sucrose percentages of the parental lines, F₁, F₂, backcrosses, F₃ progenies of selfed F₂ plants, and selfed progenies of the backcrosses obtained from the cross of lines 1 and 2 are shown in table 6.

TABLE 6.—*Frequency distributions, means, and standard deviations of sucrose percentages of parental lines 1 and 2 and of F₁, F₂, and F₃ crosses and backcrosses of 1 × 2*

PLANTS GROWN IN 1937 AND 1938

Line or cross	Year grown	Roots in sucrose-percentage class—														Total roots	Mean sucrose percentage	Standard deviation
		4	5	6	7	8	9	10	11	12	13	14	15	16	17			
		No.	No.	No.	No	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.		
1 × 2, F₁	1937									2	25	13	7			47	13.48±0.11	0 76±0.08
Check	1937							1	1	9	9	12	11	1	2	46	13.62±.21	1.42±.15
1	1938							20	73	52	4					149	12.24±.06	.71±.04
(1 × 2) × 1	1938								1	4	5	8	3			21	13.30±.23	1.04±.16
1 × 2, F₂	1938						8	52	103	124	53	8				348	12.49±.06	1.03±.04
(1 × 2)×2	1938						2	1	13	21	8					45	12.71±.13	.85±.09
2	1938						1	2	6	47	70	11				137	13.52±.07	.79±.05
Check	1938						2	6	38	59	47	14	3			169	13.07±.08	1.07±.06

F₃ PROGENIES OF SELFED F₂ PLANTS, GROWN IN 1939

1939 culture No.	Sucrose percentage of parent plant	Roots in sucrose-percentage class—														Total roots	Mean sucrose percentage	Standard deviation
		4	5	6	7	8	9	10	11	12	13	14	15	16	17			
		No.	No.	No.	No.	No	No	No.	No.	No	No.	No.	No	No	No.	No.		
32	14 0						1	1	9	11	2					24	11.43±0.19	0.91±0.13
33	13 9						1	2	9	4		1				17	11.14±.25	1.03±.18
34	13 8				1		4	4	3	3						15	9.97±.35	1.36±.25
35	13 8				1	1	4	1	1							8	9.76±.43	1 21±.30
36	13 5							3		3	1					7	11.13±.41	1.10±ed 29
37	13 3	1	3	4	5	3	1	2								19	7.70±.38	1.66±.27
38	13 3				1	4	6	6								17	9.94±.19	.79±.14
39	13 1					6	2									8	10.32±.21	.58±.14
40	13 1				1	4	3									8	10.14±.24	.68±.17
41	13 0			1	3	6	3	1								14	9 88±.30	1.12±.21
42	12 9					5	1	2								8	10.58±.26	.74±.18
43	12 8				1	3	14	8	1							27	10.22±.14	.74±.10
44	12 8					1	3	4	4	3			1			16	11.64±.38	1 54±.27
45	12 7				2	2	9	11		1						25	10.34±.21	1.04±.15
46	12 6					4	9	6								19	10.12±.17	.73±.12
47	12 5			1		2	3	4								10	8 85±.41	1 29±.29
48	12 5				1	3	3	7	4	1						19	10.62±.27	1.18±.19
49	12 4					1	1	6	3	4						15	11.40±.31	1.19±.22
50	12 2				1	2	4	5	6							18	9.59±.25	1.06±.18
51	12 1					4	7	4	3							18	10.32±.25	1 08±.18
52	12 0				2	2	7	3		1	1					16	9 17±.40	1.60±.28
53	12 0						2	11	17	5						35	10.70±.13	.79±.09
54	12 0				1		3	11	5	1						21	10 75±.30	1.36±.21
55	11 8					1	1	7	6	1	1					17	11.38±.26	1.09±.19
56	11 8			1		1	5	8	7	5	1					28	9 20±.27	1.42±.19
57	11 7				1		2	3	5	9						20	10.00±.27	1.23±.19
58	11 7					1	10	7	2							20	9.45±.15	.66±.10
59	11 6				1	1	3	7	9	2						23	10.09±.25	1.22±.18
60	11 4					3	2	4								9	9.09±.23	.70±.16
61	10 7						2	17	7							26	10 16±.10	.53±.07

SELFED PROGENIES OF BACKCROSS (1×2)×1

No.	parent	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total roots	Mean	Standard deviation
109	14 5				1			3	6	3						13	10.60±0.27	0 97±0.19
110	14 1							8	9	6						23	10.85±.16	.75±.11
111	13 0							1	9							10	11.85±.11	.34±.08
112	12 9				1	5	13	6								25	10 02±.15	.76±ish 11
113	12 5					3	6	8	1							18	10.39±.21	.87±.14
114	12 4				2	6	8	1								17	9.35±.19	.77±.13
115	12 3				1	5	22	8								36	9.91±.11	.67±.08
116	11 2					2	6	1								9	9.81±.23	.65±.15

TABLE 6.—*Frequency distributions, means, and standard deviations of sucrose percentages of parental lines 1 and 2 and of F_1, F_2, and F_3 crosses and backcrosses of 1×2*—Continued.

SELFED PROGENIES OF BACKCROSS (1×2)×2

1939 culture No.	Sucrose percentage of parent plant	Roots in sucrose-percentage class—														Total roots	Mean sucrose percentage	Standard deviation
		4	5	6	7	8	9	10	11	12	13	14	15	16	17			
		No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.		
117...	14.1					1	1	8	7	10						27	10.79±0.20	1.02±0.14
118...	14.1						1	3	8	9	3					24	11.30±.20	.97±.14
119...	13.7							1	6	1	1					9	11.03±.25	.75±.18
120...	13.6							7	12	2	1					22	10.79±.21	.97±.15
121...	13.5							1	13	6						20	11.25±.13	.56±.09
122...	13.3							10	7	10	2					29	11.06±.18	.99±.13
123...	13.1						1	4	9	8	1					23	11.16±.19	.92±.14
124...	13.1						2	11	10							23	10.29±.12	.60±.09
125...	12.8					1	1	4	5	2						13	10.43±.26	.95±.19
126...	12.8				1	6	14	6	2							29	8.96±.16	.88±.12
127...	12.8							2	10	12	4					28	11.68±.18	.98±.13
128...	12.6					1	3	10	5							19	10.02±.17	.75±.12
129...	12.4					2	2	3	1							8	9.26±.31	.86±.22
130...	12.1					2	4	11	6	1						24	9.93±.19	.94±.14
131...	11.2						1	3	3							7	10.06±.32	.85±.23

The mean sucrose percentages of the parents, the F_1, and the F_2 generation were 12.88±0.05, 12.93±0.11, and 12.49±0.06, respectively, the F_1 being reduced by 0.55 percent since the check was that much higher in 1937 than in 1938. The differences between the means of the parents, the F_1, and the F_2 in percentage sucrose was not great.

When the F_1 was backcrossed to line 1, the sucrose percentage was nearly equal to that of line 2, and the reciprocal backcross, (1×2)×2, was more nearly like line 1 in sucrose percentage. Thus the backcrosses were not in accord with expectation, and the results could not be explained on the basis of the performance of the parental lines. Apparently the genotype of the plants used in these crosses was not truly described by the mean performance of the parent lines. The fact that line 1 was relatively heterozygous has been pointed out previously.

The parental lines and the F_1 appeared to be equally variable in their frequency distributions, while the F_2 was more variable than either the parents or the F_1. The backcross (1×2)×1 was as variable as the F_2, while the difference between the standard deviations of the F_2 and the backcross (1×2)×2 approached significance.

Of the 30 F_3 families of this cross, grown in 1939, 10 appeared to be as uniform in sucrose percentage as the more uniform P_3 lines of the parents, and 20 appeared to be less uniform. The mean sucrose percentages of each group were divided equally above and below the mean of the 30 families.

After allowance was made for the variability that existed within the parental lines, it seemed evident that certain families were as homozygous as the parental lines, while others were fully as variable as the F_2 population.

The mean sucrose percentages of lines 1 and 2 grown in 1939 were 9.07 and 10.63, respectively (table 2). Eight F_3 families had a mean

sucrose percentage equal to or greater than the higher sucrose parent. One of these, culture 53, was no more variable than the more uniform selfed progenies of line 1.

Progenies produced from selfed backcross plants were more uniform than the F_2, and in many cases appeared as uniform as the original parents, which would indicate that a single generation of backcrossing had tended definitely to reduce the genetic variability of the progeny.

<div align="center">CROSS 1 × 4276</div>

Table 7 shows the frequency distributions and means of sucrose percentages of parental lines, F_1, F_2, and F_3 crosses, backcrosses, and selfed backcross progenies obtained from the cross of lines 1 and 4276.

TABLE 7.—*Frequency distributions, means, and standard deviations of sucrose percentages of parental lines 1 and 4276 and of F_1, F_2, and F_3 crosses and backcrosses of 1 × 4276*

PLANTS GROWN IN 1937 AND 1938

Line or cross	Year grown	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total roots	Mean sucrose percentage	Standard deviation
1 × 4276, F_1	1937									2	12	5				19	13.09±0.16	0.69±0.11
Check	1937							1	1	9	9	12	11	1	2	46	13.62±.21	1.42±.15
1	1938								20	73	52	4				149	12.24±.06	.71±.04
(1×4276) ×1	1938						2	5	26	13	7					53	12.31±.13	.92±.09
1 × 4276, F_2	1938					3	7	17	43	65	30	7	2			174	11.59±.10	1.33±.07
(1×4276) ×4276	1938					1	9	19	11	3						43	10.13±.09	.59±.06
4276	1938				12	62	56	11								145	8.51±.07	.86±.05
Check	1938								2	6	38	59	47	14	3	169	13.07±.08	1.07±.06

F_1 PROGENIES FROM SELFED F_2 PLANTS, GROWN IN 1939

1939 culture No.	Sucrose percentage of parent plant	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total roots	Mean sucrose percentage	Standard deviation
62	13.2				1		12	7	2							22	9.31±0.17	0.79±0.12
63	12.7		1		1	1	3	7	1							15	9.80±.50	1.92±.35
64	12.7						2	5	9	2						18	10.64±.18	.76±.13
65	12.5				1	1	4	5	5	1						17	8.88±.30	1.25±.21
66	12.4					3	6	7	3	2						21	8.65±.26	1.18±.18
67	12.4					1	1	3	2	5						12	9.62±.43	1.49±.30
68	12.1			1	2	3	7	4	7		1					25	8.46±.31	1.56±.22
69	12.1		2	2	1	4	2	4	3							18	8.39±.51	2.18±.36
70	11.9						1	4	3	1						9	9.48±.29	.87±.21
71	11.9				1		4	7								12	9.33±.27	.93±.19
72	11.8				2	5	3	7	4	1						22	10.40±.28	1.32±.20
73	11.6					6	10	1	1							18	8.84±.17	.70±.12
74	11.4			1		4	4	1	2							12	8.66±.37	1.27±.26
75	11.3				1	2	10	8	2	1			1			25	10.60±.31	1.54±.22
76	11.3		4	3	3	6	3	2								21	7.30±.36	1.65±.25
77	11.0				1	4	5	6	7		1					24	9.76±.29	1.41±.20
78	10.8					1	2	8		2						13	9.85±.32	1.14±.22
79	10.7				1	1	4		1							7	8.84±.46	1.22±.33
80	10.7			2	3	3	4			1						13	8.05±.43	1.56±.31
81	10.6				2	5		1								8	7.82±.31	.88±.15
82	10.6		1	2	2	6	3	1								15	7.73±.48	1.85±.34
83	10.6					5	6	6								17	8.96±.21	.89±.15
84	10.5			1		3	5	6	3	2						20	8.62±.28	1.25±.20
85	10.5	2	1		2	5	2	1								13	7.97±.64	2.30±.45

TABLE 7.—*Frequency distributions, means, and standard deviations of sucrose percentages of parental lines 1 and 4276 and of F_1, F_2, and F_3 crosses and backcrosses of 1 × 4276*—Continued.

SELFED PROGENIES OF BACKCROSS (1 × 4276) × 1

1939 culture No.	Sucrose percentage of parent plant	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total roots	Mean sucrose percentage	Standard deviation
182	13.4				1		3	6	7	3						20	10.22±0.27	1.20±0.19
133	13.2			1	1	1	13	10	4	1						31	9.43± .20	1.12± .14
134	12.8					2	7	13	7							29	9.65± .17	.91± .12
135	12.5						2	4	6	2						14	10.52± .21	.80± .15
136	12.4				1	2	9	7								19	9.02± .18	.77± .12
137	12.4					9	8	3								20	8.62± .15	.65± .10
138	12.3						10	12	6							28	9.88± .15	.81± .11
139	12.1				2	4	9	3	1							19	8.78± .24	1.06± .17
140	12.0				2	2	2		1							7	8.41± .47	1.25± .33
141	11.8			6	8	8	3									25	9 32± .19	.97± .14
142	11.6		2	3	6	9	3	1	2							26	7.57± .33	1.69± 23
143	11.7				1	2	7	7	3		1					21	9 56± .27	1.23± .19
144	11.2				4	6	9	9	5		2					35	9.36± .24	1.44± .17

SELFED PROGENIES OF BACKCROSS (1 × 4276) × 4276

1939 culture No.	Sucrose percentage of parent plant	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total roots	Mean sucrose percentage	Standard deviation
145	13.8						1	2	3	1	2					9	10.98±0.43	1.28±0.30
146	13.6					1	1	2	3	1						8	10.14± .49	1.37± .34
147	12.3					1	3	8	4	5						21	10.39± .23	1.07± .17
148	12.2				5	6	2	1								14	7.91± .23	.85± .16
149	11.2			2	1	4	1	1		2						11	8.52± .64	2.12± .45
150	11.0		2	2	2	2										8	6.26± .56	1.59± .40
151	10 5			4	1	4										9	6.98± .35	1.04± .25
152	10.4					5	7	9		1	1					23	9.50± .28	1.34± .20
153	10.3		5	4	4	2	2									17	6.49± .34	1 38± 24
154	9.9				1	5	10	1								18	8.51± .22	.94± .16
155	9.8		2	5	4	5	1									17	6 82± .29	1.20± .21
156	9.5			1	2	5										8	7.39± .33	.92± .23
157	9.4			1	2	5										8	7.39± .23	.65± .16
158	9.3			1	3	7	1	1								13	7.66± .29	1 03± .20
159	8.3		3	3	1		1			2						10	7.25± .91	2.87± .64

The mean sucrose percentage of the F_1 was 2.71 percent higher than the mean of the two parents. After allowance was made for the difference in sucrose percentage of the check for the 2 years, the net increase of the F_1 over the average of the two parents was 2.16 percent. The mean sucrose percentage of the parents and the mean of the F_2 could be compared directly since both progenies were grown the same year under the same conditions. The mean sucrose percentages of the parents and of the F_2 were 10.38±0.05 and 11.59±0.10, respectively. The difference (1.21±0.11) was significant. The mean sucrose percentage of the F_1 backcrossed to the parent higher in sucrose percentage was equal to the sucrose percentage of the recurrent parent.

The F_1 cross was as uniform as the parental lines, and the F_2 was significantly more variable. The backcross (1 × 4276)× 4276 was less variable and the backcross (1 × 4276)× 1 slightly more variable than the recurrent parents, but both backcrosses were less variable than the F_2.

The range of values of the F_2 generation exceeded that of the higher parent by one class and failed by one class to reach the lower limit

of the lower parent. The progeny of the backcross to the higher parent was distributed in a manner similar to the higher parent, while the backcross to line 4276 did not recover the lowest percentage sucrose class of line 4276.

Twenty-four F_3 families were grown in 1939 from selfed seed of F_2 plants. Of these, 7 appeared to be as uniform as the parental lines, while 17 were more variable.

Some of the most uniform families, e. g., culture 64, were appreciably above the average in sucrose percentage, while others, e. g., culture 81, were considerably below the average. The same was true of the variable families; certain ones were high in mean sucrose percentage (culture 75) and others were low (culture 85).

Progenies produced from selfed backcross plants were in many instances as variable as the F_2 generation, while in other instances they were as uniform as the parents. As in the case of the cross 1 × 2, there was no apparent relationship between the mean sucrose percentage of the F_3 families and the variability within the families.

The mean sucrose percentage of the 13 progenies of (1 × 4276)× 1 was 9.26 percent. This was slightly higher than the mean of the 24 F_3 progenies. The mean of the 15 selfed progenies of (1 × 4276) × 4276 was only 8.15 percent. While the variation between the progenies was relatively large, backcrossing produced a pronounced shift in sucrose percentage, on the average, in the direction of the recurrent parent.

<center>CROSS 2 × 4276</center>

The frequency distributions and means of the sucrose percentages of the parental lines, F_1, F_2, and F_3, and backcrosses of the cross 2 × 4276 are shown in table 8. Since the two lines used in this cross were relatively homozygous, as demonstrated previously, it would be expected that this cross would provide more critical data than the crosses 1 × 2 and 1 × 4276.

TABLE 8.—*Frequency distributions and means of sucrose percentage of parental lines 2 and 4276 and F_1, F_2, and F_3 crosses and backcrosses of 2 × 4276*

<center>PLANTS GROWN IN 1937 AND 1938</center>

Line or cross	Year grown	Roots in sucrose-percentage class—														Total roots	Mean sucrose percentage	Standard deviation
		4	5	6	7	8	9	10	11	12	13	14	15	16	17			
		No.	No.	No.	No.	No.	No.	No.	No.	No.	No	No.	No.	No.	No.	No.		
2×4276, F₁	1937							8	9	10	5					32	11.32±0.18	1.02±0.13
Check	1937							1	1	9	9	12	11	1	2	46	13.62±.21	1.42±.15
2 (2×4276)	1938								1	2	6	47	70	11		137	13.52±.07	.79±.05
×2 2×4276,	1938						2	4	10	26	23	10	3			78	12.33±.14	1.23±.10
F₂ (2×4276)	1938					5	40	51	27	11	1					135	10.93±.09	1.05±.06
×4276	1938				2	13	11	15	3							44	9.98±.15	1.01±.11
4276	1938			12	62	56	11	3	1							145	8.51±.07	.86±.05
Check	1938								2	6	38	59	47	14	3	169	13.07±.08	1.07±.06

TABLE 8. — *Frequency distributions and means of sucrose percentage of parental lines 2 and 4276 and F_1, F_2, and F_3 crosses and backcrosses of 2 × 4276*—Continued

F₃ PROGENIES OF SELFED F₂ PLANTS, GROWN IN 1939

1939 culture No.	Sucrose percentage of parent plant	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total roots	Mean sucrose percentage	Standard deviation
		No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.		
86	13.2							5	11	10						26	11.10±0.15	0.76±0.11
87	13.1				1		3	5	4	2						15	10.03±.31	1.19±.22
88	12.6							2	2	3						7	11 20±.39	1 04±.28
89	12.4				1	1	4	10	5							21	9 77±.25	1.13±.17
90	12.3						3	4	2	2						11	10 29±.31	1.04±.22
91	12.2			3	4	3	1	1								12	7.49±.47	1.62±.33
92	12.0			1			7	2	3							13	9.28±.33	1 19±.23
93	11.7		1		2	3	7	3	1							17	10 05±.29	1.18±.20
94	11.6			2	3	3	1	1								10	7.60±.39	1 22±.27
95	11.6						5	10	1							16	9.73±.19	.76±.13
96	11.5		2	1	6	3	1									13	7.05±.33	1.18±.23
97	11.3		1	2	4	8	5	2								22	8.60±.26	1.21±.18
98	11.3				4	1	7	1								13	8.24±.29	1 05±.21
99	11.0				3	6	11	9	3	1	1					34	9.21±.22	1.31±.16
100	11.0				1	3	8									12	8.58±.19	.64±.13
101	11.0				1	3	3									7	8.13±.32	.84±.22
102	10 9				3	7	3									13	8.05±.21	.75±.15
103	10 5		1	1	1	5	2	1								11	7.88±.40	1 33±.28
104	10.5			1	3	6	3	4								17	8 29±.29	1.18±.20
105	10 4				7	5	3	2								17	7 99±.22	.90±.15
106	10.3		1	2	4	5	5	2								19	7 79±.35	1.53±.25
107	10.2			1	1	6	6	6	3	1						24	9.08±.29	1.43±.21
108	10.1		2		1	8	2									13	7 39 36	1 32± 26

SELFED PROGENIES OF BACKCROSS (2×4276)×2

1939 culture No.	Sucrose percentage of parent plant	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total roots	Mean sucrose percentage	Standard deviation
160	13.0				3	6	7	3								19	8.46±0.22	0 98±0.16
161	12.9				1	3	6	1								11	8.56±.21	.71±.15
162	12.9	3			2	1	10	2	2							20	7.22±.42	1 87±.30
163	12.3				4	2	6	8		1						21	8.99±.27	1.22±.27
164	12 2						3	7		1	1	1				12	10.12±.35	1 22±.25
165	12.1				2	8	2	2	1							15	8.47±.25	.98±.18
166	11.8			1	1	3	6	4								15	8.77±.32	1 24±.23
167	11 5			1	1	7	12	4	2		1					28	8 97±.25	1 30±.17
168	11 5			1		6	6	8	2							23	9 05±.25	1.18±.17
169	11 4				1	3	9	6								19	9 04±.18	80±.13
170	11 4				2	3	8	3	7	4		1				28	8.90±.33	1.72±.23
171	11.4				1	2	4	1	4	2						14	8.81±.39	1 44±.27
172	10.8		1	2	5	1	1									10	6.65±.34	1.07±.24

SELFED PROGENIES OF BACKCROSS (2×4276)×4276

1939 culture No.	Sucrose percentage of parent plant	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total roots	Mean sucrose percentage	Standard deviation
173	11.4	1	4	7	7	4	4									27	6.70±0.26	1 34±0.18
174	11.0			1	2	3	3	1								10	8.05±.39	1.25±.28
175	10.9				7	13	3	3								26	9.03±.19	.95±.13
176	10.9			2	5	3	1									11	6.25±.24	.79±.17
177	10.8	1			4	2		1								8	7.95±.71	2.00±.50
178	10.8			2	3	4	4	2								15	7.93±.30	1.18±.22
179	10 7				2	1	5	4	1							13	9 01±.32	1.16±.23
180	10 7		2		4	9	2	3								20	6.98±.26	1.15±.18
181	10 5				2	7	5	1								15	7.20±.19	.73±.13
182	10.4				3	11	9	2	1				e			26	7.51±.19	.96±.13
183	9.8			1	5	2	1									9	6.42±.24	.71±.17
184	9.5			1	5	5	2									13	6.51±.24	.85±.17
185	9.4		1	2	3	2	2									10	6.20±.44	1.39±.31
186	9.2	4	1		8	8	3	1	1							26	6 39±.30	1.52±.21
187	9.1	1			3	6	1									11	6 38±.36	1.19±.25
188	8.8			5	7	18	8									38	6.79±.16	.96±.11

The F_1 generation of the cross 2×4276 grown in 1937 tested 11.32 ± 0.18 percent sucrose compared with 11.02 ± 0.5 percent as an average of the two parents grown in 1938. Since the check variety averaged 0.55 percent higher in 1937 than in 1938, it may be concluded

that the F_1 was not significantly different from the mean of the parents. The F_2 generation averaged 10.93 ± 0.09 percent sucrose as compared with an average of 11.02 ± 0.05 for the parental lines.

Each of the backcrosses might be expected to have a mean sucrose percentage equal to three-fourths of the sucrose percentage of the recurrent parent plus one-fourth of the sucrose percentage of the nonrecurrent parent. Each backcross closely approached the expected value, exceeding it only 0.06 and 0.22 percent in the backcrosses to the higher and lower testing parent, respectively.

The F_2 and both backcrosses were more variable than the parents. Six of the F_3 families were as uniform in sucrose percentage as the original parents, while many of the families were fully as variable as the F_2 generation. The variability among the progenies from the selfed backcross mothers was very similar to the variability found among the F_3 families. It appeared that lines no more variable than the parents can be isolated relatively easily in the F_3. Only 2 of the 23 F_3 progenies had a mean sucrose percentage as great as the high-sucrose parent. One of these, culture 86, also was no more variable than the parents. No F_3 lines were found in which the sucrose percentage was as low as in the low-sucrose parent, if culture 29 in line 4276 is disregarded (table 2).

When the F_1 was backcrossed to the high-sucrose parent, line 2, the mean of the selfed backcross progenies was 8.62 percent, a slightly lower value than the average (8.82 percent) obtained from the 23 F_3 families. Backcrossing to the low-sucrose parent resulted in a mean of 7.21 percent for the selfed backcross progenies, which was decidedly lower than the mean of the F_3. In cultures 176, 183, and 184, the mean sucrose percentage was essentially the same as that of the low-sucrose parent and the variability was very little or no greater.

CORRELATION BETWEEN SUCROSE PERCENTAGE OF PARENT AND MEAN OF THE PROGENY

Correlation coefficients were calculated for sucrose percentages of roots of parent plants selected from segregating populations and the means of their progenies. Correlation coefficients are shown in table 9.

TABLE 9.—*Coefficients of correlation of sucrose percentages of roots of F_2 plants and means of F_3 progenies and between backcrosses and means of the selfed progenies*

Cross	Generations correlated	Comparisons	r [1]
		Number	
1 × 2	F_2 and F_3	30	0.1757
1 × 4276	do	24	.4425
2 × 4276	do	23	.6859**
(1 × 2) × 1	Backcrosses and progeny	8	.5141
(1 × 2) × 2	do	15	.5619*
(1 × 4276) × 1	do	13	.4672
(1 × 4276) × 4276	do	15	.7099**
(2 × 4276) × 2	do	13	.0459
(2 × 4276) × 4276	do	16	.5519*
Average			.4659**

[1] *Exceeds 5-percent level of significance; **exceeds 1-percent level of significance.

The correlation coefficients between the sucrose percentage of F_2 mother roots and the mean of the F_3 progenies were not significant in crosses 1×2 or 1×4276. In cross 2×4276 the correlation coefficient was highly significant. Sucrose percentages of the roots of the backcrossed plants were significantly correlated with the mean sucrose percentage of the progenies in three cases, and nonsignificantly correlated in three others. All correlation coefficients were positive.

Rider (5) described a method of using χ^2 for testing the homogeneity of a group of correlation coefficients. Applying this test for homogeneity, it was found that $\chi^2 = 9.51$ and $P = 0.30$, indicating that the correlation coefficients were homogeneous and could be considered as arising from a single population. An average correlation coefficient of 0.4659 was obtained by transforming r to z as outlined by Fisher (3), which exceeded the value required for odds of 99 : 1. Selection of roots with a high percentage of sucrose, from segregating populations, would aid in the isolation of F_3 progenies high in sucrose percentage.

DISCUSSION

Data obtained from the three crosses studied indicated that the F_1 was approximately equal to the mean of the two parents in sucrose percentage. The only exception was in the case of cross 1×4276, in which the F_1 was higher than the mean of the parents, probably due to the fact that the genotype of the plants in line 1 used in the cross was not truly represented by the mean of the line. Line 1 was the most variable of the three lines used in these crosses. In the F_2, the mean sucrose percentage was slightly but not significantly higher than the mean of the parents. When the parents did not differ greatly in sucrose percentage, the extremes of both parental types were recovered in the distribution of the F_2 sucrose percentages.

F_3 families were obtained in which the mean sucrose percentage was similar to that of the high-sucrose parent, and several of these families were no more variable than the parental lines. In two cases, reciprocal backcrosses produced progenies with mean sucrose percentages which approached that of the recurrent parent. This was especially true of the cross between the two most uniform parental lines, 2 and 4276. From the selfed backcrosses progenies were obtained that were like the recurrent parent in mean sucrose percentage and uniformity.

Since F_3 families as uniform in sucrose percentage as the parent families were obtained frequently in all three crosses, it might appear that this quality was inherited in a relatively simple manner. On the other hand, these less variable families had mean sucrose percentages that in general fell within the range of the P_3 families of the parent higher in sucrose percentage. In no instance were found F_3 families of uniform sucrose percentage that fell in the range of P_3 familes of line 4276. Thus it appears that it may not be easy to recover in F_3 the sucrose type of the parents and that the actual number of genetic factors influencing sucrose percentage may be fairly great. It seems doubtful if there are genetic factors that influence only sucrose percentage; it is more probable that the physiologic processes of the plant are controlled genetically and that these processes in turn control the production and storage of sucrose. It also seems probable, as shown by Pack (4), that many factors which influence principally morpho-

logic characters of the plant may influence to a greater or less extent the sucrose percentage of the beet.

The ability of a sugar beet to store sucrose appears to depend upon the interaction of many genetic factors, some of which exert an important influence and some a relatively unimportant influence upon its expression. From the data obtained from these crosses, it seems that it should be possible to retain the desirable sucrose percentage of one inbred when crossed with other inbreds having a lower sucrose percentage but excelling in other characteristics.

SUMMARY

A study was made of the inheritance of sucrose factors in crosses between inbred lines of beets differing in percentage of sucrose. The average sucrose percentage of the F_1 and F_2 generations was approximately equal to the mean of the parents, being very close in the cross of the two most uniform lines. F_3 progenies and progenies of selfed backcrosses were obtained that were no more variable than the parental inbreds. Some of these progenies were as high in mean sucrose percentage as the high-sucrose parent, while others were significantly lower. When the parental lines differed widely in sucrose percentage, no F_3 progenies or progenies of selfed backcrosses were obtained that were as uniform as the parents and as low in mean sucrose percentage as the low-sucrose parent. It appeared that relatively homozygous lines of desirable sucrose percentage could be obtained comparatively easily. The ability to store sucrose was found to be inherited in a quantitative manner and to be dependent upon the interaction of several genetic factors.

The correlation between the sucrose percentage of mother beets selected from segregating populations and the means of their inbred progenies was found to be highly significant. Roots with a high percentage of sucrose, selected from segregating populations, were found to produce, as an average, F_3 progenies high in sucrose percentage.

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MATHEMATICAL MODEL OF EMBRYO ABORTION IN PHILLIPS CLING PEACHES [1]

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INTRODUCTION

Embryo abortion as indicated by a change in the color of the embryo and of the integuments of the ovule is closely associated with certain phenomena of development of peaches such as the gumming of the Phillips Cling.[2] Such evidence of embryo abortion is easy to observe but it may have taken place after the abortion itself had occurred. Thus it may be extremely difficult, if not impossible, to determine the time of abortion of the ovule and embryo by direct observation. It is nevertheless important to know the time and intensity of the tendency to abort both for reasons of practical control and theoretical connections with other related phenomena such as pit hardening and gumming.

It is possible to observe the frequency distributions of the lengths of normal embryos at intervals throughout the growing season and the frequency distribution of the lengths of the aborted embryos at harvest time. From these data it is possible to deduce the probability of abortion as a function of time. The probability function of abortion refers to the proportion of the total number of aborted embryos for the season that will occur in any interval of time. The probability of gumming can also be observed. Since abortion of embryos and gumming are so closely associated in Phillips Cling, we should find that the probability of gumming is much the same as the probability of abortion but may be shifted in point of time. Data are not now available for checking, except roughly, the agreement of the probability functions of abortion and gumming.

The purpose of this paper is to derive a mathematical model of embryo abortion on the basis of certain plausible assumptions. If the mathematical model agrees closely with the observed facts, then the assumptions may be believed to approximate the truth, and the model may be studied to shed further light on the occurrence of aborted embryos. For instance, from the mathematical model, we can compute when the susceptible period for abortion begins; when the probability of abortion is greatest; when any given percent of the abortions will occur; the relations of abortion to pit hardening, gumming, and split pit; and the like.

DEVELOPMENT OF MODEL

Let us assume that the lengths of normal embryos are distributed, at any time, according to a Pearson's type III curve, and that abortion does not cause a change in the measured lengths of the embryos.

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(173)

Let

$p(t) =$ probability of abortion at time, t

$m(t) =$ mean length of normal embryos at time, t

$\sigma(t) =$ standard deviation of the lengths of the normal embryos at time, t

$\alpha(t) =$ third moment of the frequency distribution of the lengths of the normal embryos divided by the standard deviation cubed at time, t

Then the joint frequency distribution of the aborted embryos with respect to time, t, and length, s, is

$$\frac{p(t)}{\sigma(t)\sqrt{2\pi}} \, y_0(t) \left(1+\frac{\alpha(t)}{2\sigma(t)}(s-m(t))\right)^{\frac{4}{[\alpha(t)]^2-1}} e^{-\frac{2(s-m(t))}{\alpha(t)\sigma(t)}} \tag{1}$$

on the basis of the stated assumptions. Time, t, is measured in weeks, and length, s, is measured from the beginning of the range. $y_0(t)$ is a function of t such that the integral of (1) from 0 to ∞ with respect to s is $p(t)$. $y_0(t)$ is approximately

$$\left(1-\frac{[\alpha(t)]^2}{48}\right)$$

Since the integral of $p(t)$ from the time of the first abortion to harvest-time with respect to t is 1 the total volume under the surface (1) is 1.

Let

$t_0 =$ time of abortion of the first embryos

$t_\infty =$ harvest time

$F_0(s) =$ observed frequency distribution of the lengths of the aborted embryos at harvest time

$F(s) =$ theoretical frequency distribution of the lengths of the aborted embryos at harvest time

$_0M_i =$ moments about the mean of $F_0(s)$

$_0M'_i =$ moments about the beginning of the range of $F_0(s)$

$_tM_i =$ moments about the mean of $F(s)$

$_tM'_i =$ moments about the beginning of range of $F(s)$

Then

$$_tM_i' = \int_{t_0}^{t_\infty} p(t)\left[\int_{-\frac{2}{\alpha(t)}}^{\infty} \frac{1}{\sqrt{2\pi}} \, y_0(t) \, (\sigma(t)s+m(t))^i \right.$$
$$\left. \cdot \left(1+\frac{\alpha(t)}{2}s\right)^{\frac{4}{[\alpha(t)]^2}-1} e^{-\frac{2s}{\alpha(t)}} \, ds \right] dt \tag{2}$$

If we assume that $_tM_i' = {_0M_i'}$ then (2) serves to determine $p(t)$. Suppose that $p(t)$ is determined by its first four moments. Then we need

$$_0M_1' = \int_{t_0}^{t_\infty} p(t)m(t)dt \tag{3}$$

$$_0M_2' = \int_{t_0}^{t_\infty} p(t)([\sigma(t)]^2+[m(t)]^2)dt \tag{4}$$

$$_0M_3' = \int_{t_0}^{t\infty} p(t)\,([\sigma(t)]^3\alpha(t) + 3[\sigma(t)]^2 m(t) + [m(t)]^3)\,dt \tag{5}$$

$$_0M_4' = \int_{t_0}^{t\infty} p(t)\left\{[\sigma(t)]^4\left(3 + 3\frac{[\sigma(t)]^2}{2}\right) + 4[\sigma(t)]^3 m(t)\alpha(t) + 6[\sigma(t)]^2[m(t)]^2\right.$$
$$\left. + [m(t)]^4\right\}dt \tag{6}$$

Let

$$M_1(t) = m(t) \tag{7}$$

$$M_2(t) = [\sigma(t)]^2 + [m(t)]^2 \tag{8}$$

$$M_3(t) = [\sigma(t)]^3\alpha(t) + 3[\sigma(t)]^2 m(t) + [m(t)]^3 \tag{9}$$

$$M_4(t) = [\sigma(t)]^4\left(\frac{6 + 3[\alpha(t)]^2}{2}\right) + 4[\sigma(t)]^3 m(t)\alpha(t)$$
$$+ 6[\sigma(t)]^2[m(t)]^2 + [m(t)]^4 \tag{10}$$

and assume that

$$M_i(t) = a_{i0} + a_{i1}t + a_{i2}t^2 + a_{i3}t^3 + a_{i4}t^4,\ (i = 1,\ 2,\ 3,\ 4) \tag{11}$$

Let u_i' $(i = 1,\ 2,\ 3,\ 4)$ be the moments of $p(t)$ about some convenient point, then the u_i' are determined from

$$_0M_i' = a_{i0} + a_{i1}u_1' + a_{i2}u_2' + a_{i3}u_3' + a_{i4}u_4',\ (i = 1,\ 2,\ 3,\ 4) \tag{12}$$

The moments of $p(t)$ about its mean, denoted by u_i $(i = 1,\ 2,\ 3,\ 4)$, are then easily determined. In most cases $p(t)$ can then be satisfactorily represented by the corresponding Pearson curve.

APPLICATION TO OBSERVED DATA

The data on the constants of the frequency distributions of the normal embryos and the functions (7), (8), (9), and (10) for an orchard in Sutter County, Calif., for the season of 1939 are given in table 1. These data were supplied by Professor L. D. Davis and were collected as described [3] by him.

TABLE 1.—*Data for illustrating the mathematical model of peach abortion, Sutter County, Calif., 1939*

Date	Number observed	$m(t)$	$s(t)$	$\alpha(t)$	$M_2(t)$	$M_3(t)$	$M_4(t)$
May 23	200	4.56	1.26	0.27	22.38	117.08	648.2
May 30.	200	8.50	1.88	.00	75.78	704.25	6,789.7
June 6..	150	12.24	2.04	—.86	153.98	1,979.28	25,899.9
June 13.	200	15.22	1.49	—.52	233.87	3,625.34	56,658.7
June 20	200	15.32	1.37	—.46	236.58	3,680.72	57,669.1
June 30	200	16.23	1.04	—.17	264.49	4,327.66	71,087.0
July 7...	200	16.14	1.06	.00	261.62	4,258.87	69,620.0

The detailed frequency distributions of the normal embryos at dates throughout the season are given in table 2. These distributions are skewed in one direction, then symmetrical, then skewed in the other

[3] DAVIS, L. D. SIZE OF ABORTED EMBRYOS IN THE PHILLIPS CLING PEACH. Proc. Amer. Soc. Hort. Sci. 37: 198–202, illus. 1939.

direction, and then symmetrical again.   Pearson's type III curves
are the simplest that can take account of these changes in form.

TABLE 2.—*Frequency distribution of normal embryos at specified dates,*
Sutter County, Calif., 1939

Class intervals (millimeters)	Frequency distributions on—						
	May 23	May 30	June 6	June 13	June 20	June 30	July 7
0.0-0.9							
1.0-1.9	2						
2.0-2.9	14						
3.0-3.9	44						
4 0-4.9	65	2					
5.0-5.9	48	5					
6.0-6 9	21	27	4				
7.0-7 9	5	34	3				
8 0-8 9	1	42	5				
9.0-9.9		28	9				
10.0-10 9		29	9	2	1		
11.0-11 9		10	21	4	4		
12.0-12.9		6	34	9	5		
13 0-13.9		2	37	18	16	4	4
14.0-14 9			20	39	36	15	19
15.0-15.9			8	58	71	52	56
16 0-16.9				51	17	75	75
17.0-17.9				17	11	48	38
18 0-18.9				1	6	6	8
19.0-19 9				1			
Total	200	200	150	200	200	200	200

The observed frequency distribution of the lengths of aborted
embryos at harvesttime is given in table 3.   It should be noted that
the centers of the class intervals in tables 2 and 3 are at the $x.45$
millimeter marks, where x stands for the integers 0–19.

The constants for $F_0(s)$ are

$$_0M_1' = 10.71 \qquad _0M_2 = 8.36 \qquad _0M_3 = -22.42$$
$$_0M_4 = 246.89 \qquad _0\beta_1 = 0.86 \qquad _0\beta_2 = 3.53$$

The $F_0(s)$ for Sutter County is unusually skewed, as was pointed out
by Davis, and the corresponding probability distribution for gumming
is not available.   The $p(t)$ obtained for these data may be expected
to differ somewhat from the probability distributions of gumming
obtained for other years when $F_0(s)$ was found to be symmetrical.

In computing (7), (8), (9), and (10) the polynomials were made to
take on the first three values and the average value of each of the last
two pairs of values in table 1.   This procedure was used because $p(t)$
weights the first values much heavier than the later values.   Usual
least-squares technique gives too much weight to the less important
later intervals unless polynomials of higher degree are used.   But
higher degree polynomials have a tendency to change erratically in
the important time interval.   The origin for t is taken to be June 13.
The reference date for these data is May 22.

The equations for determining the u'_i are

$$1.1628u_1' - .8076u_2' + .0382u_3' + .0375u_4' = -3.9518$$

$$46.7243u_1' - 17.1908u_2' - .6923u_3' + 1.0173u_4' = -93.1255$$

$$1{,}032.9630u_1' - 239.6403u_2' - 37.2680u_3' + 14.3985u_4' = -1{,}725.5516$$

$$20{,}422.3878u_1' - 2{,}474.3933u_2' - 988.2735u_3' + 121.1640u_4' = -29{,}491.4488$$

TABLE 3.—*Frequency distribution of the lengths of aborted embryos at harvesttime Sutter County, Calif., 1939*

Class interval (millimeters)	Number Observed	Class interval (millimeters)	Number observed
1.0–1.9	4	10.0–10.9	78
2.0–2.9	3	11.0–11.9	71
3.0–3.9	16	12.0–12.9	117
4.0–4.9	10	13.0–13.9	86
5.0–5.9	17	14.0–14.9	42
6.0–6.9	18	15.0–15.9	5
7.0–7.9	28	16.0–16.9	2
8.0–8.9	40		
9.0–9.9	59	Total	596

Whence

$$u_1' = -1.02 \qquad u_2' = 4.20$$
$$u_3' = 1.56 \qquad u_4' = 27.46$$

and

$$u_2 = 3.15 \qquad u_3 = 12.26 \qquad u_4 = 56.74$$
$$\beta_1 = 4.81 \qquad \beta_2 = 5.72 \qquad \kappa = -1.20$$

The constants of $p(t)$ call for a Pearson curve of type 1 which is U-shaped. This means that $p(t)$ is heavy on each end and light in the middle. A U-curve cannot be considered as a satisfactory representation of $p(t)$ since it is known that $p(t)$ becomes zero at the ends of its range. A probability distribution that has the required characteristics and approximately the desired moments was constructed by trial and error and is given in table 4. For comparison the probability distribution of gumming for the same orchard but for the season of 1936 is given. The main difference between the two distributions is the hump on the tail of the $p(t)$ for 1939. This hump causes the skewness of $F_0(s)$ for 1939.

Since $u_1' = -1.02$, the mean of $p(t)$ is 1 week before June 13. The end of the range is not definitely determined by the moments of $p(t)$, but from table 4 it appears that the start of abortion must coincide very closely with the beginning of pit hardening. The fact that 2 percent is given in the first class interval of $p(t)$ does not mean that abortion starts at the beginning of that week but that it starts sometime within the week. If the class marks of the probability distribution of gumming were shifted a day or so the first parts of the two distributions would be even more nearly alike.

TABLE 4.—*Comparison of p(t) for Sutter County, Calif., 1939, and the probability of gumming, Sutter County, 1936*

Period from beginning of range (weeks)	Percent of total aborted embryos [1]	Percent of total gummed fruit [2]	Period from beginning of range (weeks)	Percent of total aborted embryos [1]	Percent of total gummed fruit [2]
1	2.0		7	3.0	1
2	50.0	41	8	6.0	1
3	25.8	32	9	1.2	1
4	9.0	15			
5	2.0	7	Total	100.0	100
6	1.0	2			

[1] Aborting distribution: $u_1' = 0.11$, $u_2 = 3.24$, $u_3 = 12.28$, $u_4 = 57.02$.
[2] Gumming distribution: $u_2 = 1.71$, $u_3 = 4.23$, $u_4 = 23.40$.

DISCUSSION

The usual situation is for the frequency distributions of the lengths of aborted embryos to be symmetrical and approximately normal. In such cases the probability distribution of abortion can be adequately represented by a Pearson curve of type 1_1 of limited range which approaches the J type. Usually abortion starts at the time the pits begin to harden, reaches a maximum within the first week, and soon trails away to a very low level. Gumming starts about 11 days later and parallels the occurrence of abortion.

Sometimes, however, the frequency distributions of the lengths of aborted embryos are badly skewed as in the present example or are very flat and perhaps bimodal as for the University Farm data for the season 1939.[4] In such cases, $p(t)$ is two-humped and very difficult to represent adequately by the first four moments, although a fairly good notion of $p(t)$ can be obtained by trial and error. The $p(t)$ for Sutter County, 1939, shows a very high incidence of abortion at the time of pit hardening and a small secondary peak of abortions about 6 weeks later. This second peak is not associated with pit hardening in point of time and should be investigated. It is not known whether this second peak in abortions is reflected in the occurrence of gumming. In the University Farm 1939 data the secondary peak must be much more important than in the Sutter County 1939 data.

It is regretted that frequency distributions of lengths of normal embryos at intervals throughout the season, the frequency distribution of the lengths of aborted embryos at harvesttime, and the probability distribution of gumming are not all available for the same orchard in the same season. Since such data are not available the above statements must be considered as only tentative and subject to revision on the accumulation of more data.

It is suggested that the functions of $m(t)$, $\sigma(t)$, and $\alpha(t)$ be more carefully determined by shortening the interval of time between observed frequency distributions of normal embryos and that the possibility of a major cause of embryo abortion late in some seasons be investigated.

SUMMARY

A mathematical model of embryo abortion in Phillips Cling peaches has been constructed. Observed facts seem to indicate the efficacy of the proposed model. The probability distribution of abortion may be one-humped or two-humped. One hump is always associated with pit hardening. The gumming of Phillips Cling starts about 11 days after pit hardening and hence occurs about 11 days after abortion begins. The probability of gumming parallels the probability of abortion at least when $p(t)$ is one-humped and perhaps so or nearly so when $p(t)$ is two-humped.

[4] See footnote 3, p. 175

ROOT ROT OF RANUNCULUS ASIATICUS CAUSED BY PYTHIUM DEBARYANUM [1]

By C. M. TOMPKINS, *assistant plant pathologist*, and JOHN T. MIDDLETON, *junior plant pathologist, California Agricultural Experiment Station* [2]

INTRODUCTION

A destructive root disease of Persian buttercup (*Ranunculus asiaticus* L.) was first observed in commercial field plantings at Inglewood and Pacific Palisades, Calif., during the winter of 1937–38. Subsequently the disease has also been found in Santa Cruz, San Mateo, and San Francisco Counties. The losses are reflected not only in decreased field stands of plants, but in a smaller cut-flower crop and yield of tubers. Because of its economic importance, an investigation of this disease was conducted during the past four seasons, the results of which are briefly recorded in this paper. A preliminary note has previously been published. [3]

SYMPTOMS OF THE DISEASE

Ranunculus plants, whether produced from seeds or tubers, appear to be highly susceptible to root rot infection when grown in the field. The most striking symptoms of the disease consist of a general wilting followed by rapid collapse and death of the plant. The roots, tubers, stems, and petioles may be invaded. The roots and tubers of infected plants are dark brown, water-soaked, and flaccid. The stem plate is discolored and dark, but usually not water-soaked or flaccid. Occasionally the petioles become infected, in which event the diseased parts are dark brown, frequently exhibiting necrotic streaks which are 1 to 3 mm. wide and about 30 to 60 mm. long, extending outward from the base of the petiole and parallel to its long axis. In the field, plants are most commonly affected when 6 to 8 inches high, but, under experimental conditions, plants of all ages appear to be highly susceptible. The disease occurs on widely different soil types, ranging from coarse sand to heavy clay, and is favored by excessive rainfall or irrigation, poor drainage, cool weather, and crowding of plants.

THE CAUSAL FUNGUS, *PYTHIUM DEBARYANUM*

Isolations made from diseased material on water or malt-extract agars have consistently yielded a fungus which has been identified as *Pythium debaryanum* Hesse. The four isolates of the fungus studied, representing two districts in southern and two in central California where the disease occurs, are readily cultivated on potato-

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dextrose, malt, corn-meal, and other agars, on which they develop a copious, dense, aerial mycelium. The asexual and sexual reproductive organs are sparingly produced in the surface growth but develop rather profusely intramatrically. Both types of fruiting bodies are abundant in the diseased tissues of tubers of infected plants. Perhaps the best medium for observing the development of the reproductive structures is plain water agar. On this substrate, the sporangia are usually spherical, thin and smooth-walled, and acrogenous; numerous intercalary sporangia are produced which may be spherical or barrel-shaped. Sporangia germinate either by the production of zoospores in a vesicle or by several germ tubes. Oogonia are similar to sporangia and have an average diameter of 22.2μ. Antheridia are usually plural, varying in number from one to six,

FIGURE 1.—Root rot of Persian buttercup: *A*, Symptoms produced on young Persian buttercup plant by *Pythium debaryanum* 12 days after inoculation in the greenhouse; *B*, noninoculated control.

predominantly two to three per oogonium, monoclinous and diclinous, when monoclinous arising some distance below the oogonium and not adjacent to it. One fertilization tube is usually produced by each antheridium applied to the oogonial wall. The oospores are spherical and smooth-walled, with a mean diameter of 18.2μ. Germination of oospores has not been observed.

The temperature-growth relations of the fungus have been determined for two of the four isolates. They are: Minimum, $1°$ C., optimum, $28°$, and maximum $37°$.

All four isolates of the fungus proved pathogenic to healthy ranunculus plants grown from seeds and tubers. Inoculum was prepared by growing the fungus on sterilized cracked wheat in 8-inch test tubes. When ready for use, this was added to autoclaved soil contained in 6-inch pots in such a manner as to avoid injuring the root system of the young plants. Sterile cracked wheat was added to the pots serving as controls. All pots were heavily watered each day in order to keep the soil very moist. The incubation period

ranged from 11 to 25 days, but all infected plants died within a day or two after the foliage commenced to wilt (fig. 1). Of 20 plants inoculated with each isolate, none escaped infection, while the 20 control plants remained healthy. The fungus was reisolated from each infected plant and proved to be identical with the original isolates. When tested in parallel series, the reisolates again proved highly pathogenic. In these tests, the infected plants exhibited symptoms identical with those of naturally infected plants. The appearance of the root system of artificially infected plants, as contrasted with healthy roots, is shown in figure 2.

Although *Pythium ultimum* Trow and *P. irregulare* Buis. are commonly associated with root rots of various herbaceous ornamentals

FIGURE 2.—Root rot of Persian buttercup: *A, B, C*, Symptoms produced on the roots of young Persian buttercup plants by *Pythium debaryanum* 12 days after inoculation in the greenhouse; *D*, noninoculated control.

in California, they have not been found associated with this disease. Inoculation experiments have shown that *P. ultimum* is strongly pathogenic to *Ranunculus* while *P. irregulare* is weakly pathogenic but likewise capable of inducing disease. The symptoms produced by these two species are similar to those produced by *P. debaryanum*.

EXPERIMENTAL HOST RANGE

In studies on the host range, one isolate of the fungus from southern California was used along with the no-wound technique described for the pathogenicity tests. Healthy plants to be inoculated had four to six leaves. Reisolations were made from all experimentally infected plants, and the reisolate of the fungus from a particular host was then tested by inoculation into healthy specimens of that host.

Since it is generally believed that *Pythium debaryanum* is cosmopolitan in its attacks, a large variety of plants including many of those grown in rotation with *Ranunculus* were experimentally inoculated. The experimental host range proved to be very limited and includes Iceland poppy (*Papaver nudicaule* L.), columbine (*Aquilegia caerulea* James), fibrous-rooted begonia (*Begonia semperflorens* Link and Otto), butterflyflower (*Schizanthus pinnatus* Ruiz and Pav.), and cucumber (*Cucumis sativus* L.). If seeds had been sown in experimentally infested soil the host range might have been extended. If this technique were employed the results would be indicative of the ability of *P. debaryanum* to cause damping-off and would not indicate its potentialities as a root rotting agent of more mature plants. Infection of these hosts, characterized by sudden wilting without change in color of the foliage and water-soaked lesions on the fibrous roots, occurred in 11, 18, 27, 9, and 17 days, respectively.

No infection was obtained in 41 species of plants representing 37 genera in 23 families, as follows:

Aizoaceae:
    Iceplant (*Mesembryanthemum crystallinum* L.)
Amaranthaceae:
    Cockscomb (*Celosia argentea* L. var. *cristata* Kuntze)
Campanulaceae:
    Canterbury-bells (*Campanula medium* L.)
Caryophyllaceae:
    Sweet-william (*Dianthus barbatus* L.)
    Carnation (*Dianthus caryophyllus* L.)
Chenopodiaceae:
    Spinach (*Spinacia oleracea* L.) var. Bloomsdale
Compositae:
    Transvaal daisy (*Gerbera jamesonii* Hook. var. *transvaalensis* Hort.)
    China-aster (*Callistephus chinensis* Nees) var. Giant Branching White, wilt resistant
    French marigold (*Tagetes patula* L.)
    African marigold (*Tagetes erecta* L.)
    Gaillardia pulchella Foug. var. *picta* Gray
    Hybrid cineraria (*Senecio cruentus* DC.)
Cruciferae:
    Cabbage (*Brassica oleracea* L. var. *capitata* L.) var. Winter Colma
    Cauliflower (*Brassica oleracea* var. *botrytis* L.) var. February
    Radish (*Raphanus sativus* L.) var. White Icicle
    Dames violet (*Hesperis matronalis* L.)
    Annual stock (*Matthiola incana* R. Br. var. *annua* Voss) var. Fiery Blood Red
Dipsacaceae:
    Mourning bride or pincushion flower (*Scabiosa atropurpurea* L.)
Labiatae:
    Flowering sage (*Salvia farinacea* Benth.)
Leguminosae:
    Broadbean (*Vicia faba* L.)
Malvaceae:
    Hollyhock (*Althaea rosea* Cav.)
Onagraceae:
    Godetia grandiflora Lindl.
Papaveraceae:
    California poppy (*Eschscholtzia californica* Cham.)
    Oriental poppy (*Papaver orientale* L.)
Polemoniaceae:
    Phlox drummondii Hook.
Ranunculaceae:
    Poppy anemone (*Anemone coronaria* L.)
    Love-in-a-mist (*Nigella damascena* L.)
    Rocket larkspur (*Delphinium ajacis* L.)

Ranunculaceae (*continued*):
    Hybrid delphinium (*Delphinium cultorum* Voss)
    Meadowruc (*Thalictrum dipterocarpum* Franch.)
Resedaceae:
    Mignonette (*Reseda odorata* L.)
Rosaceae:
    Geum chiloense Balb.
Scrophulariaceae:
    Foxglove (*Digitalis purpurea* L.)
    Snapdragon (*Antirrhinum majus* L.)
    Pentstemon or beardtongue (*Pentstemon barbatus* Nutt.)
Solanaceae:
    Tomato (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey) var. Early
        Santa Clara Canner
    Tobacco (*Nicotiana tabacum* L.) var. Turkish
Tropaeolaceae:
    Garden nasturtium (*Tropaeolum majus* L.)
Umbelliferae:
    Celery (*Apium graveolens* L.) var. Golden Self Blanching
Verbenaceae:
    Garden verbena (*Verbena hybrida* Voss)
Violaceae:
    Pansy (*Viola tricolor* L.)

DISCUSSION

In California, Persian buttercup plants are grown commercially in the field from seeds and tubers for seed, tuber, and cut-flower production. When grown for cut flowers, growers generally prefer to plant seeds, which are usually drilled in rows in August. The young plants, which commence to flower early in January if not infected, are frequently invaded by the fungus in the December preceding, or soon after the winter rains commence. When grown on poorly drained land, the mortality may reach 50 percent or more. Since limited tests of various strains of seeds and tubers from different sources failed to disclose any evidence of resistance to the disease, control is dependent upon the selection of well-drained sites.

SUMMARY

A destructive root disease of Persian buttercup (*Ranunculus asiaticus*) is described.

The disease is prevalent in commercial plantings in four coastal counties of California. It is favored in its development and spread by excessive moisture, poor drainage, cool weather, and crowding of plants.

The chief symptoms of the disease consist of a general wilting followed by rapid collapse and death of the plant. The roots, tubers, stems, and petioles may be affected.

Pythium debaryanum has been consistently isolated from infected plants and has proved pathogenic in greenhouse tests. The morphology and temperature relations of the fungus are discussed.

The incubation period for the disease ranges from 11 to 25 days.

In greenhouse tests, the fungus proved pathogenic to young plants of Iceland poppy, columbine, fibrous-rooted begonia, butterflyflower, and cucumber.

Persian buttercup seedlings were also experimentally infected with *Pythium ultimum* and *P. irregulare*. The symptoms were similar to those produced by *P. debaryanum*.

PANTOTHENIC ACID IN THE NUTRITION OF THE PIG [1]

By E. H. HUGHES [2]

Animal husbandman, California Agricultural Experiment Station

INTRODUCTION

As early as 1938, Hughes [3] reported that the addition of a rice bran filtrate to a purified diet containing thiamin, riboflavin, and nicotinic acid resulted in more rapid and economical growth in the pig. This product was a good source of what was then known as the filtrate factor. In the spring of 1940 sufficient quantities of synthetic optically inactive pantothenic acid were available to determine whether this substance was necessary for growth and physical well-being in the pig. [4]

EXPERIMENTAL DATA

Two experiments were conducted. The experimental procedure, equipment, and pigs used were similar to those employed in studies formerly reported [5] on the factors of the vitamin-B complex. The basal diet and salt mixture were similar to, but not identical with, those used previously. The basal ration consisted of beet sugar 81 percent, casein (purified) 15, salt mixture 4, cod-liver oil (Squibb's tested) 10 cc. per pig weekly, thiamin 3 mg., riboflavin 3 mg., nicotinic acid 15 mg., and vitamin B_6 5 mg. per 100 pounds of pig daily. In these diets where synthetic pantothenic acid (calcium pantothenate) was included it was fed at a level of 14.7 mg. per 100 pounds of pig daily, or 6.8 mg. of pantothenic acid.

The salt mixture was composed of the following salts: Calcium phosphate (dibasic) 40 percent, sodium chloride 20, calcium carbonate 11, ferric chloride 3, copper sulfate 0.2, manganese sulfate (anhydrous) 0.5, zinc oxide 0.1, magnesium citrate 15, cobaltous acetate 0.1, potassium chloride 10, and potassium iodide 0.1

In the first experiment, which was begun September 24, 1940, and continued until November 19, 1940, only five pigs were available. Two pigs were fed the basal diet without pantothenic acid, and three pigs were fed the same diet to which 6.8 mg. of pantothenic acid was added per 100 pounds of pig daily. Growth of these two groups is presented graphically in figure 1, *A*.

The pigs in group 1 grew very slowly while those in group 2 gained an average of 0.82 pound per head daily, a very good gain for pigs of their weight and age.

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The second experiment covered a period of 84 days, beginning November 26, 1940, and ending February 18, 1941. There were 10 pigs in this test, 5 in each group. An examination of the growth curves in figure 1, *B*, shows that the growth of pigs in this experiment was similar to that in the first experiment. The pigs in group 1 grew slowly, but those in group 2, fed pantothenic acid, made an average daily gain of 0.74 pound.

Among the outward manifestations of a deficiency of pantothenic acid in these studies were an early decrease in appetite and slow growth of the pigs, and an inability to move about in a normal manner within about a month after being placed on a deficient diet. The pigs apparently lost their sense of equilibrium and correlation; they "goose-stepped" and often fell; they became thin and emaciated; their hair became dry and finally after a period of about 70 days two of the pigs lost most of their hair; the feces were often watery, and in some instances blood was passed. The photographs (fig. 2) supplement the description given.

Four of the pigs were autopsied. One died on the seventy-seventh day and another soon after the second experiment was concluded; the other two were killed. The autopsies showed gastritis in each, which included a reddened area on the floor of the stomach about the size of a normal hand. Scattered throughout this area were hemorrhagic spots ranging in size from 1 to 2 mm.

FIGURE 1.—Growth curves of pigs fed a basal diet deficient in pantothenic acid as compared with those of pigs fed 6.8 mg. of pantothenic acid per 100 pounds of body weight daily: *A*, experiment 1; *B*, experiment 2.

There was some inflammation in the large intestine of each, and in one there were many abscesses (fig. 2, *D*). On the skin of the two pigs that lost most of their hair, there were many reddened areas about 7 mm. in diameter.

DISCUSSION

Chick et al.[6] and Hughes suggest that pigs fed diets sufficient in nicotinic acid, thiamin, and riboflavin need other factors of the vitamin-B complex. Further evidence of the necessity for one or both of factors 1 (vitamin B $_6$) and 2 (the filtrate factor) was pre-

[6] CHICK, HARRIETTE, MACRAE, THOMAS FOTHERINGHAM, MARTIN, ARCHER JOHN PORTER, and MARTIN, CHARLES JAMES. THE WATER-SOLUBLE B-VITAMINS OTHER THAN ANEURIN (VITAMIN B₁), RIBOFLAVIN AND NICOTINIC ACID REQUIRED BY THE PIG. Biochem. Jour. 32: 2207-2224, illus. 1938. (See also reference cited in footnote 3, p. 185.)

sented by Hughes.[7]   In a recent publication Wintrobe et al.[8] described symptoms in pigs which seem similar to those observed in this investigation in pigs deficient in pantothenic acid.

Goose-stepping in pigs has been reported from many sections of the country.   It has been noticed at this station among pigs fed a heated diet composed largely of corn, wheat middlings, and casein (unpublished
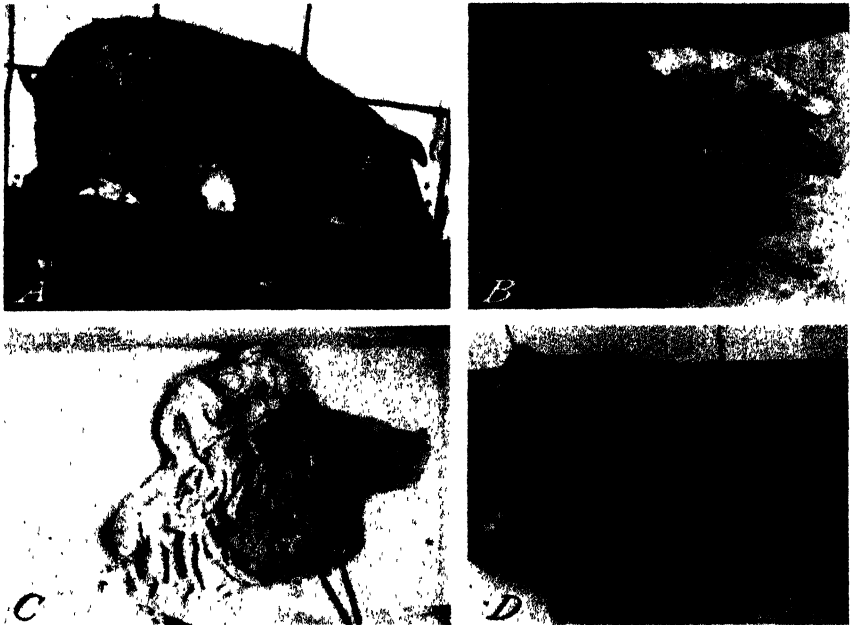
FIGURE 2.—Results of feeding pigs a basal diet deficient in pantothenic acid: *A*, Goose-stepping; *B*, loss of hair; *C*, stomach with reddened area indicating gastritis; *D*, abscesses in the large intestine.   The matted condition of the hair was caused by the dirt sticking to it.

data) and among pigs fed a diet deficient in factor 2 (filtrate factor)[7].   Such pigs walked poorly and several became crooked in their hocks.   This condition is associated with a deficiency of pantothenic acid.

SUMMARY

Young pigs fed a diet deficient in pantothenic acid developed subnormal appetites, grew slowly, became thin and emaciated, showed a lack of normal coordination, "goose-stepped," some lost their hair, and at autopsy showed gastritis and involvement of the large intestine.   It is therefore concluded that pantothenic acid is necessary for normal growth and well-being in the young growing pig.

[7] See first reference footnote 5, p. 185.
[8] WINTROBE, MAXWELL M., MILLER, JOSEPH L., JR., and LISCO, HERMANN. THE RELATION OF DIET TO THE OCCURRENCE OF ATAXIA AND DEGENERATION IN THE NERVOUS SYSTEM OF PIGS. Johns Hopkins Hosp. Bul. 67: 377-404. illus. 1940.

THE POTASSIUM REQUIREMENT OF GROWING PIGS [1]

By E. H. Hughes, *animal husbandman*, and N. R. Ittner, *associate in animal husbandry, California Agricultural Experiment Station*

INTRODUCTION

This paper reports the results of one of a series of studies of purified diets for pigs now being carried on at the California Experiment Station at Davis. During the summer of 1940 considerable difficulty was experienced with the purified diets that were being fed except the control diet in which skim-milk powder was included. In previous experiments where a rice-bran filtrate was fed at a 5-percent level no serious difficulties were encountered. One of the diets fed was cane sugar 82 percent, purified casein 15 percent, salt mixture 3 percent, thiamin 3 mg., riboflavin 3 mg., calcium pantothenate 14.7 mg. (pantothenic acid 6.8 mg.), nicotinic acid 15 mg., and vitamin B_6 5 mg. per 100 pounds of pig daily, and 10 cc. of cod-liver oil per pig weekly. The salt mixture had been used previously. On this ration the pigs soon lost their appetites and ceased to grow. They scoured to some extent, certain individuals vomited, others seemed weak in their hindquarters, and one was unable to rise. Similar pigs fed a diet of cane sugar 68 percent, purified casein 5 percent, skim-milk powder 25 percent, salt mixture 2 percent, and 10 cc. of cod-liver oil per pig weekly gained over 40 pounds in 4 weeks and seemed perfectly normal.

It was evident that some deficiency was responsible for the trouble. From the information at hand, it appeared that whatever was lacking was water-soluble, for when a rice-bran filtrate was fed at a 5-percent level the results were good. Skim-milk powder fed at a 25-percent level also provided something that was necessary. Since casein was present in the basal diet, it was expected that the necessary nutrient would be present in whey. At the time that these diets were being fed to pigs, the same diets were being fed to rats. Whey was accordingly fractionated and fed to growing rats, with the result that when the inorganic salts of whey were added to the deficient diet a decided improvement was noticed during the first week. When the salt mixture that was being used was compared to the inorganic salts in milk it was found that the potassium content of the salt mixture was probably much too low. Potassium chloride therefore was substituted in the rat diets for the inorganic salts of milk, and the results in growth were the same as with the inorganic salts of milk.

In the meantime, the pigs were in such a bad state of health that it appeared they would die. Whey powder was added to the diet and a decided improvement took place both in their appetites and in their growth. Later potassium was substituted for the whey powder with equally good results. With the facts established that the salt mixture which had been used was not adequate because of its lack of potassium and that potassium was necessary for the pig, the next step was to determine the minimum potassium requirement of the pig.

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MATERIAL AND METHODS

Four groups of five pigs each, uniform in size and weighing about 50 pounds at the beginning of the experiment, were used in this study. They were fed and watered in steel troughs. Their quarters were similar in size and shape and had concrete floors and wooden partitions. The wooden partitions were covered with small-mesh wire to keep the pigs from chewing the boards. The pigs had access to inside and outside pens.

The basal diet consisted of beet sugar 81 percent, purified casein 15 percent, salt mixture 4 percent, cod-liver oil 10 cc. per pig weekly, thiamin 3 mg., riboflavin 3 mg., nicotinic acid 15 mg., pantothenic acid 6.8 mg., and vitamin B_6 5 mg. per 100 pounds of pig daily. The salt mixture was composed of calcium phosphate (dibasic) 45 percent,
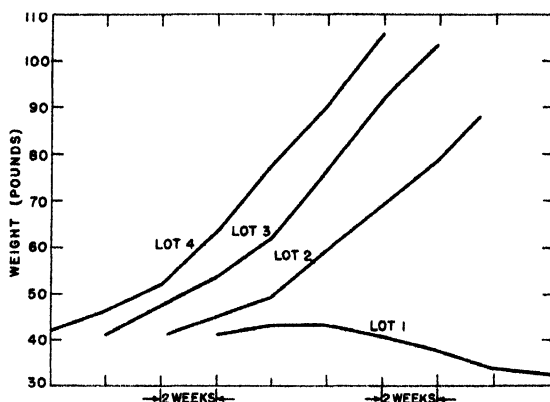
FIGURE 1.— The effect of the level of potassium intake upon the growth of pigs: Lot 1 received no potassium; lots 2, 3, and 4 received 2.25, 4.5, and 9.5 gm. of potassium chloride per 100 pounds of body weight daily.

sodium chloride 22 percent, calcium carbonate 12 percent, ferric chloride 3 percent, magnesium citrate 17 percent, cobaltous acetate 0.1 percent, copper sulfate 0.2 percent, manganese sulfate (anhydrous) 0.5 percent, zinc oxide 0.1 percent, potassium iodide 0.1 percent.

The following diets were fed, beginning November 26, 1940:

Lot 1.—Basal (no potassium except the potassium iodide in the salt mixture).
Lot 2.— Basal + 2.25 gm. of potassium chloride per 100 pounds of pig per day.
Lot 3.—Basal + 4.5 gm. of potassium chloride per 100 pounds of pig per day.
Lot 4.—Basal + 9.5 gm. of potassium chloride per 100 pounds of pig per day.

The sugar, casein, and salt mixture were weighed and mixed thoroughly at the beginning of the experiment. The vitamins were weighed weekly, put in water solution, and mixed with the feeds for the various lots. The amount of gain and the feed consumption of the previous week made it possible to determine the amounts that would be consumed the following week. The potassium was weighed weekly and handled like the vitamins. The feed mixtures were kept in paper-lined garbage cans, the feed for each lot in a different one. Separate buckets were used to feed each lot. The pigs were weighed individually.

EXPERIMENTAL DATA

The growth rate of the four lots is presented graphically in figure 1. The pigs in lot 1 gained slightly for a time then lost weight. Those in lot 2 gained throughout the feeding period of 12 weeks, though not so rapidly as those in lots 3 and 4. One pig in lot 2 gained slightly at first, then lost weight and finally died. The pigs in lots 3 and 4 gained at practically the same rate. The gains of all lots were slow at first because of the change from a normal grain diet to a highly purified diet and because of the relatively small daily food consumption of small pigs.

At the end of 4 weeks all the pigs in lot 1 had to be helped to rise. Three had died. The first indication of a deficiency was reduced appetite; soon thereafter the pigs would stand and squeal and become unsteady on their legs. Later they would be found on the floor and would be unable to get up without help. Once on their feet they could move around reasonably well and were able to fight for their feed at the trough. Two of the pigs lived throughout the experiment. However, they became very thin and emaciated, their appetites were poor, and they remained unsteady on their legs.

At autopsy the three that died early were found to be in fairly good condition and they were not thin. In each case there was feed in the stomach with little or no feed or feces in the small or large intestine. In one there was a large congested area on the wall of the stomach. In the other two there were ulcers in the stomach, some small, others measuring about 15 mm. in diameter, and the large intestine showed inflammation and some hemorrhagic areas. The two pigs that remained on the experiment were killed at its conclusion and autopsied. They were exceedingly thin and emaciated and showed some inflammation of the walls of the stomach. It would appear, therefore, that diets deficient in potassium affect the mucous membrane of the stomach and large intestine.

DISCUSSION

In this study a highly purified diet was fed to growing pigs with and without sufficient potassium. Other workers have reported the requirements of this element for the rat and the chick. Osborne and Mendel [2] found the growth of rats to be fairly satisfactory on a potassium intake of about 0.03 percent provided the sodium intake was adequate. Miller [3] stated that the growth of rats can be greatly retarded by reducing the potassium content below approximately 0.1 percent. He found too that substituting sodium for potassium failed to produce normal growth in rats.

Heppel and Schmidt [4] reported a negative balance when lactating mothers (rats) were fed a potassium level of 0.14 percent in the diet.

Recently Ben Dor,[5] working with growing chicks, reported that at least 0.17 percent of potassium was required if approximately maximal growth was to be expected and that more than 0.13 percent was required to prevent heavy mortality.

[2] OSBORNE, THOMAS B., and MENDEL, LAFAYETTE B. THE INORGANIC ELEMENTS IN NUTRITION. Jour. Biol. Chem. 34: 131-139, illus. 1918.
[3] MILLER, H. G. POTASSIUM IN ANIMAL NUTRITION. II. POTASSIUM IN ITS RELATION TO THE GROWTH OF YOUNG RATS. Jour. Biol. Chem. 55: 61-78, illus. 1923.
[4] HEPPEL, LEON A., and SCHMIDT, CARL L. A. STUDIES ON THE POTASSIUM METABOLISM OF THE RAT DURING PREGNANCY, LACTATION, AND GROWTH. Calif. Univ. Pubs., Physiol. 8: 189-205, illus. 1938.
[5] BEN DOR, BEN-AMI. REQUIREMENT OF POTASSIUM BY THE CHICK. Soc. Expt. Biol. and Med. Proc. 46: 341-343. 1941.

The results reported here indicate that the requirement for growing pigs lies between 2.25 gm. (lot 2) and 4.5 gm. (lot 3) of potassium chloride, or in terms of potassium, 1.18 gm. and 2.36 gm. respectively, per 100 pounds of pig daily.

The growth rate of the pigs in lot 2 was considerably less than that of the pigs in lot 3, while the gains of lots 3 and 4 were similar. The additional potassium given to the pigs in lot 4 over that of lot 3 appeared to be unnecessary. These figures stated in terms of the diet fed are 0.08 percent and 0.15 percent. Inasmuch as the pigs in lot 2 gained less rapidly than those in lots 3 and 4 and one pig in this lot died, 1.18 gm. of potassium per 100 pounds of pig daily or 0.08 percent of the diet is not sufficient; therefore, the requirement is probably near 2.36 gm. of potassium per 100 pounds daily or 0.15 percent of the ration. These figures are in fair agreement of those of Miller, Heppel, and Schmidt for the rat and Ben Dor for the chick.

SUMMARY

It has been shown in these studies that potassium is necessary for normal growth in the young pig.

Under the conditions of this experiment the requirement of potassium chloride for the young pig lies between 2.25 gm. and 4.5 gm. per 100 pounds of pig daily. In terms of potassium this amounts to 1.18 gm. and 2.36 gm. respectively, and as percentage of the diet it amounts to 0.08 and 0.15 percent. Since 1.18 gm. of potassium per 100 pounds of pig daily or 0.08 percent of the diet was not sufficient, 2.36 gm. per hundredweight of pig daily or 0.15 percent of the diet is considered the minimum requirement for young growing pigs.

VARIATIONS IN THE DOWN COLOR OF WHITE LEGHORN CHICKS AND THEIR ECONOMIC INSIGNIFICANCE [1]

By W. F. LAMOREUX, *assistant professor of poultry husbandry*, and F. B. HUTT, *professor of animal genetics, Department of Poultry Husbandry, New York (Cornell) Agricultural Experiment Station*

INTRODUCTION

Although adult White Leghorns are all pure white (except for an occasional female with salmon breast, or a male with "brassiness" or with red on the shoulders), the down color of the chicks varies from an almost pure white in some individuals to a rich, golden yellow in others. Some poultrymen consider that the paler chicks are less vigorous and less desirable than the richly colored ones, but no actual test to determine the validity of such beliefs has hitherto been reported. Therefore, when striking differences in down color were observed in two strains of White Leghorns being selected at this laboratory for high and low requirements of riboflavin, a study was undertaken to find (1) the genetic basis for these variations, and (2) the relation, if any, of down color in the chick to such economically important characters as body size, viability, age at sexual maturity, and capacity for egg production.

MATERIAL AND METHODS

BASES OF SELECTION

In the first year of selection in Single-Comb White Leghorns for high and low requirements of riboflavin, it became evident that most of the chicks from dams considered to have a high requirement of that vitamin were darker in color than those in the "low" line. Because riboflavin is yellowish, it seemed possible that the intensity of yellow color in the down might be related to the chick's quantitative requirement of that vitamin. For that reason, all chicks in both lines were classified according to color. Later evidence showed that riboflavin had little or nothing to do with the color of the down feathers. Moreover, possibly because of difficulties inherent in the procedures used, the selection practised proved to be ineffective, and the two strains did not really differ in requirement of riboflavin. For that reason, they are hereafter designated in this paper merely as the "dark" and "light" strains.

Selection of breeders in 1936, 1937, and 1938 was based upon previous tests of their ability to reproduce when on diets deficient in riboflavin. Color of down was not considered. However, in 1939, chicks

of the dark strain were obtained from pullets belonging to the two darkest (dams') families hatched in the previous year. Similarly, chicks of the light strain were hatched from pullets in the two lightest (dams') families of the year before.

Although eggs were incubated in two different forced-draught incubators, during any one period all eggs were placed together in the same machine, so that any changes in humidity or other environmental conditions could not affect the chicks of one strain more than those of the other.

CLASSIFICATION

For purposes of this study, the chicks were classified with respect to down color, as follows:

Dark.—Golden brown color on the back of the neck, across the shoulders, and along both sides of the breast.
Medium.—Intermediate between dark and light.
Light.— Very light cream color, sometimes with a greenish cast. White is rare.

Typical representatives of these three grades, reproduced by color photography, are shown in plate 1. In the two strains studied, there seemed to be a clear, natural distinction between dark chicks and those classified as medium or light. The medium and light chicks were therefore combined in one group so that the populations might be compared with greater ease. When this is done, any population can be briefly described by stating merely the proportion of dark chicks that it contains. On the other hand, in unselected control populations, and in the F_1 progeny from certain crosses, there was a uniform gradation in the intensity of color from dark to light. The chicks of medium color are considered separately in some of the analyses reported in this paper in order to compare either the extremes in pigmentation, or their effects. Chicks of the dark, medium, and light shades are quite indistinguishable later on when fully feathered.

A classification of colors using only three grades followed by a combination, for some purposes, of the two lighter shades may seem inadequate. It must be remembered, however, that the range of color in White Leghorns is not great. A comparison of the color standards used in the present work with those used by Warren $(11)^2$ in classifying Rhode Island Reds shows that the range of intensity of color was less in the writers' dark, medium, and light chicks than in the lightest three of his five grades.

GENETIC ANALYSIS

DOWN COLORS IN THE ORIGINAL STRAINS

The proportions of chicks in the dark and light strains that were graded as dark are shown in table 1.

It should be noted that in 1937, even though color of down had not been considered as a basis for selection of breeding stock, there was such a difference between the strains that 94.4 percent of the chicks in one strain graded as dark, while only 2.8 percent of those in the other were so classified. So far as is known, the selection practiced could have had no effect upon the segregation of genes for color. It is assumed, therefore, that the original differences between the two strains were the result of chance segregation of the genes responsible.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 205
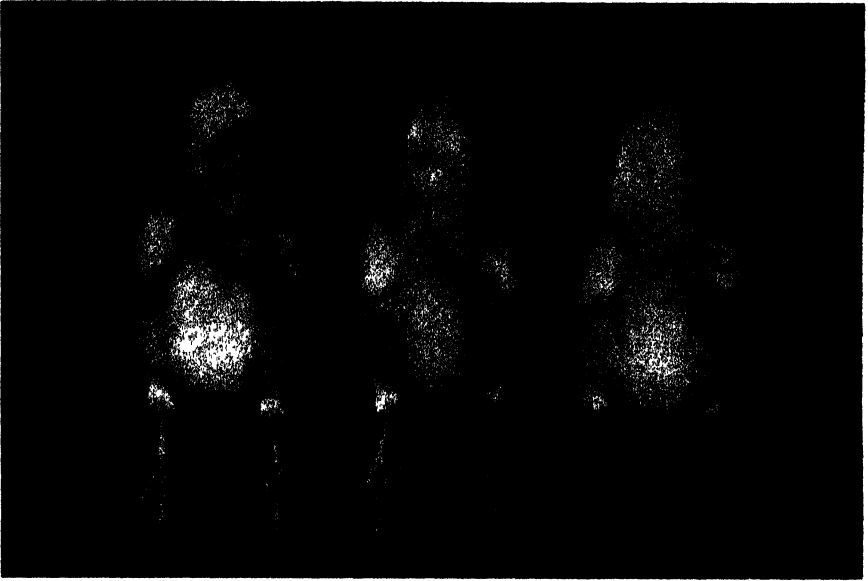
Typical representatives of dark, medium, and light colored White Leghorn chicks. In the two strains studied there seemed to be a clear, natural distinction between dark chicks and those classified as medium or light. For this reason the two lighter shades were sometimes combined to facilitate comparison.

The rapid differentiation of the dark and light strains, without selection for color, suggests that if more than one gene is responsible for the differences in down color, they must be few in number.

TABLE 1.—*Proportions of chicks having dark down in the dark and light strains and in controls, 1936–40*

Year	Dark strain		Light strain		Unselected controls	
	Chicks classified	Chicks with dark down	Chicks classified	Chicks with dark down	Chicks classified	Chicks with dark down
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*
1936	42	61. 9	257	19. 5		
1937	124	94 4	428	2. 8	827	27 7
1938	91	85. 7	296	12. 5		
1938S [1]	52	92. 0	45	4 0		
1939	14	100 0	44	4 5		
1940	68	83. 8	217	3. 7		

[1] A selected population including only chicks of the 2 darkest and 2 lightest families

After 1938, selection of breeders from the two darkest and two lightest families in the dark and light strains, respectively, effectively maintained the proportion of dark chicks at a high level in one strain and at a low level in the other. The data in table 1 show that neither strain "bred true" for its predominant shade of down, but even the exceptions of 14.3 and 12.5 percent (in 1938) are not great if one considers the difficulty of making the somewhat arbitrary classifications necessary in dealing with shades of chick down. Most of the variation occurred in a few families. Others, particularly those having darkest color, bred true to type.

In table 1, the populations labeled "1938S" include all chicks in the two darkest and two lightest families (of dams) in the dark and light strain respectively. Exceptions to the rule of color in these families were fewer than in the unselected 1938 populations from which they were taken. These few selected birds provided the parents of the 1939 generation, and these in turn were used to produce the chicks of 1940.

To determine the frequency of dark and light downs in an unselected population, 827 chicks representing four different strains of Leghorns were classified in 1937. Of these, 27.7 percent were dark, 59.6 percent medium, and 12.7 percent light. This distribution is in marked contrast to the uniformity of the dark and light strains and serves to emphasize the degree of differentiation between them.

RECIPROCAL CROSSES OF DARK AND LIGHT STRAINS

Because of the evidence, already discussed, that only a few genes were responsible for the differentiation of the dark and light strains, and the additional fact that both were breeding fairly true to type, it seemed probable that crosses between the two strains would shed some light on the genetic bases for the difference. Reciprocal crosses were made so that the effects of any sex-linked genes involved would be evident in the F_1 generation. Several different matings spread over 2 years were used for these crosses. An F_2 generation was hatched and also backcrosses of the F_1 to both the dark and light lines. The results in these various types of matings are shown in figure 1.

For simplicity, the nature of the dark and light strains, F_1, F_2, and backcross populations is shown in figure 1 merely by giving the proportion of each population that was classified as dark. The same dark females that produced the dark-strain chicks shown in figure 1 were also mated with males of the light strain (but at a different time) to produce part of the F_1 chicks considered in that figure. Similarly, the light-strain chicks and the F_1 generation from light females $\times$ dark males are all progeny of the same dams. The difference between the first and third columns in figure 1 shows, therefore, that the light-down males yielded only about two-thirds as many dark chicks as did the dark-down males when both kinds were mated to the same dark-
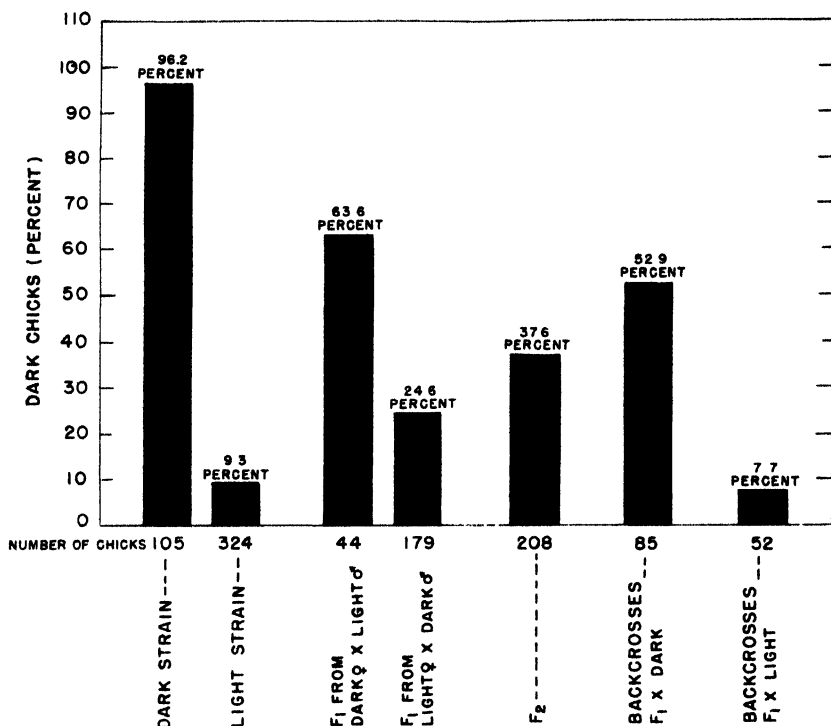
FIGURE 1.-- Proportion of darkly-colored chicks in the dark and light strains, in the F_1 generations, in the F_2 generations, and in backcrosses.

down females. In the reciprocal cross, use of the dark males raised the proportion of dark chicks from 9.3 percent within the light strain to 24.6 percent in the F_1 generation.

To determine whether or not the progeny of dark males are significantly different from those of light-strain males when both are mated to the same hens, the results of such matings with 15 hens representing both strains were tabulated. The proportion of dark-colored chicks among the progeny of each of the 30 different matings was calculated in percent, and then converted to equivalent angles for statistical treatment as suggested by Snedecor (*10, p. 381*). An analysis of variance then showed that there were significantly more dark-colored chicks from the dark- than from the light-strain males, with odds of more than 100 : 1 that the difference was not due to chance.

In the two F_1 populations, the proportions of chicks with dark down were intermediate between those in the parent strains. Because of the discrepancy between the proportions of dark chicks in these two populations (63.6 and 24.6 percent), it is doubtful whether the dark and light downs can be simple alleles differentiated only by a single autosomal gene. In that case, one would expect about the same proportion of dark chicks in each population.

The discrepancy between the two progenies from reciprocal crosses suggests that sex-linked genes were exerting some influence on the down color in the F_1 generation. However, if this were so, one would expect a sharp difference between the two F_1 populations of females (since in the fowl the female is the heterogametic sex) with respect to color of down.

This is illustrated by assuming a condition of incomplete dominance, but assigning to the character light down, the gene symbol L, and to dark down the symbol l. From reciprocal crosses, only one color of female could then be expected from each of the crosses as follows:

Dark female $(l-) \times$ light male $(LL) \longrightarrow$ only light females $(L-)$.
Light female $(L-) \times$ dark male $(ll) \longrightarrow$ only dark females $(l \quad)$.

In the first reciprocal cross (table 2, No. 1), there were obtained 21 dark females and 27 light ones. In the second cross, a great excess of light-colored females was found (table 2, No. 2) where only dark ones would be expected if the character were controlled by a sex-linked gene. These results show, therefore, that sex-linked genes have little control, if any, over the color of down, and that the differences in pigmentation of chicks obtained from reciprocal crosses must be explained in some other way.

TABLE 2.— *The colors of male and female chicks from reciprocal crosses between the light and dark strains*

Recip- rocal cross No.	Mating	Males		Females	
		Dark	Medium and light	Dark	Medium and light
		Number	*Number*	*Number*	*Number*
1	Dark females × medium or light males	36	22	21	27
2	Medium or light females × dark males	17	57	16	61

In this same mating (table 2, No. 2), there was a corresponding excess of light chicks among the males, so that whatever influence the dams exerted upon the color of the progeny was approximately equal in both sons and daughters. The fact that less than 22 percent of the progeny of light dams bore dark down, while more than 53 percent of the progeny of dark dams did so (table 2), strongly suggests that some maternal influence is exerted upon the color of down in both sexes. The mechanism responsible for such an effect is unknown.

In the F_2 population as a whole, 38 percent of the 208 chicks had dark down. However, among those from a medium-down F_1 male, son of a dark dam, the proportion of dark chicks was only 23 percent, while the the F_2 progeny of a dark F_1 male from a light-strain female 60 percent were dark.

The numbers of chicks in table 2 differ from those of similar matings shown in figure 1 because different numbers of breeders were used.

Some hens were used in reciprocal matings (table 2) that were not also used for matings within their strains, as was required for figure 1.

RELATION OF DOWN COLOR TO SEX

In chicks classified as having medium down color, the ratio of males to females was almost identical with the normal expectation of 1:1 (table 3). However, among the dark chicks there was a marked excess of males, and among the light ones a corresponding excess of females (table 3).

TABLE 3.—*Distribution of down colors in relation to sex, showing an excess of males in the dark chicks and an excess of females in the light ones, 1937–39*

Population	Year	Dark		Medium		Light	
		Males	Females	Males	Females	Males	Females
		Number	*Number*	*Number*	*Number*	*Number*	*Number*
Unselected controls	1937	165	64	257	236	40	65
Light strain	1937	6	6	135	133	63	85
	1938	29	8	106	111	13	29
F₂ generation	1939	47	21	43	52	5	13
Total		247	99	541	532	121	192
		Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Proportion in each color class		71	29	50	50	39	61

Down colors and sex ratios in the dark strain are omitted from table 3. Populations of that strain contained approximately equal numbers of males and females, but, since 84 percent or more of the chicks in that strain were of the darkest shade, with only 16 percent or less distributed through the medium and light shades, it could yield little information about the relation of down color to sex. The unselected control population, the light strain, and the F₂ generation showed more variability in down color. In all of these, the predominance of males among the dark chicks and of females among the light ones was consistent (table 3).

It may be noted that the populations included in table 3 contain 909 males, but only 823 females. This deficiency of females below expectation, and the discrepancies between the numbers in table 3 and those for the unsexed chicks of figure 1, probably resulted from the fact that the sex of all males was recorded when they were segregated at about 8 weeks of age, whereas the sex of females was not recorded until they were housed at about 5 months of age. By that time, their numbers had been diminished somewhat by predators. However, there is little likelihood that the females lost differed in color of down from those that survived (see table 5). For that reason, the deficiency of females cannot have affected the proportions of dark, medium, and light females given in table 3, although it has undoubtedly reduced the number in each of these classes.

Since families of dark chicks in the selected strains were differentiated from medium chicks more sharply than were the light-colored ones, there is some basis for combining the medium and light chicks in one class. When this is done, the totals for the populations given in table 3 are as follows: In males, 247 dark : 662 medium and light; in females, 99 dark : 724 medium and light.

Application of the chi square test to this distribution yields a value for χ^2 of 62 and for P of <0.001. This shows that the proportion of dark chicks among males is significantly higher than in females.

RELATION OF DOWN COLOR TO CHARACTERS OF ECONOMIC IMPORTANCE

To verify or disprove the belief of some poultrymen that the paler Leghorn chicks are less desirable than the dark ones, females of the dark and light strains, previously classified according to down color,
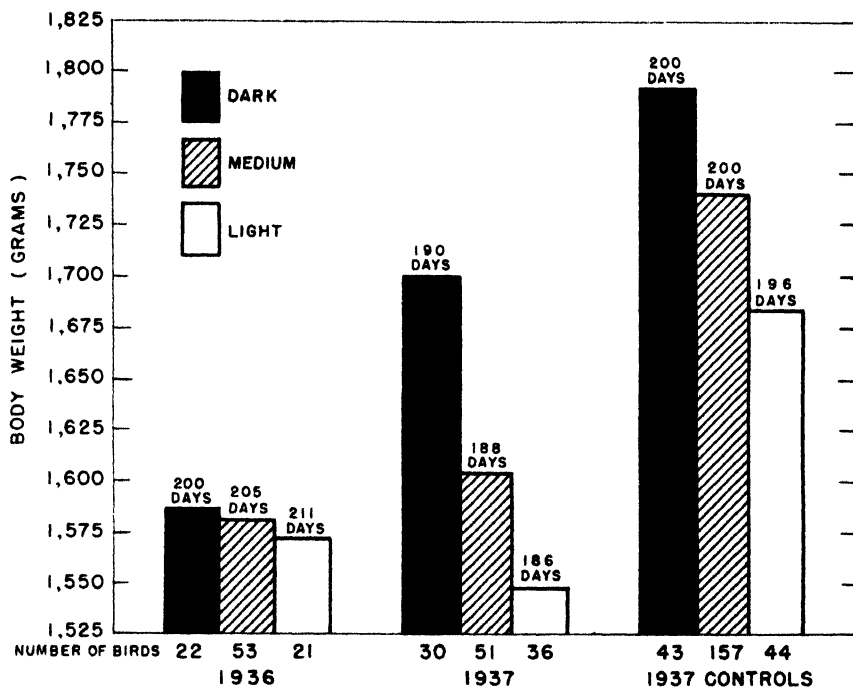
FIGURE 2.-- Body weights and ages at first egg for dark, medium, and lightly colored chicks, 1936 and 1937. The numbers on the tops of the columns are the mean ages at first egg in each group.

were kept in 1936 and 1937 for measurements of their viability, pro·ductivity, body size, and age at sexual maturity. The results, presented in tables 4 to 6 and in figure 2, are not given separately for each strain, but rather for dark, medium, and light chicks as classes, regardless of the strain from which they originated. In addition, 359 chicks of the unselected control population of 1937 were utilized for similar comparisons.

BODY WEIGHT AND AGE AT SEXUAL MATURITY

It was not possible to take weights regularly of all the available birds, but, to determine whether or not the paler chicks were smaller at hatching or developed into "runts" at an early age, two lots of chicks were weighed at hatching and again at 11 days of age. The

mean weights in these two lots (table 4) show that light chicks were no smaller than the others at 11 days of age, were larger than the medium-colored ones at hatching, and smaller than the dark ones at hatching by differences that could hardly be significant with the numbers involved. Weight of the chick at hatching is highly correlated with the weight of egg. Since light and dark chicks do not differ in size, it seems improbable that the down color could be related in any way to size of egg.

TABLE 4.—*Relation of down color of chicks to weight at hatching and early growth*

| Lot No. | Age | Mean weight [1] of chicks with indicated down color | | | Lot No. | Age | Mean weight [1] of chicks with indicated down color | | |
		Dark	Medium	Light			Dark	Medium	Light
	Days	*Grams*	*Grams*	*Grams*		*Days*	*Grams*	*Grams*	*Grams*
1	{ 1	36 5	33 2	33.8	2	{ 1	36.7	35.4	36 2
	{ 11	65.0	63 1	65 0		{ 11	68 5	57 3	69 1

[1] 15 to 75 chicks per group

Weights at sexual maturity in three separate lots of birds were consistently highest for the dark chicks and lowest for the light ones (fig. 2). Chicks of medium down color had body weights intermediate between those of the other two classes. The consistent association of larger size with darker down in all three classes looks significant. However, further study of these data by the analysis of covariance (*10*) showed that in the birds of 1936 and in the 1937 controls there existed no significant differences in body weight when the groups were adjusted to a common basis with respect to age at first egg. A value for P of <0.05 was obtained with the data from 1937. This value is often used to show that a significant difference does exist, but in view of the fact that significant differences were not found in the other two populations, one is forced to conclude that no important relationship between color of down and body weights exists in these data.

VIABILITY

Mortality from hatching to 160 days of age, when pullets were put in winter quarters, did not differ significantly or consistently in dark, medium, and light chicks of three separate lots (table 5).

TABLE 5.—*Independence of down color and viability in White Leghorn pullets to 160 days of age, 1936 and 1937*

| Year | Total chicks in all 3 down-color classes | Mortality to 160 days of age | | | Total pullets in all 3 down-color classes | Mortality from 160 days t[1] Jan 23 [1] | | |
		Dark down	Medium down	Light down		Dark down	Medium down	Light down
	Number	*Percent*	*Percent*	*Percent*	*Number*	*Percent*	*Percent*	*Percent*
1936	175	14	18	18	142	10	12	26
1937	177	23	21	21	139	9	15	11
1937, controls	359	11	12	9	250	[2] 42	[2] 46	[2] 38

[1] This period had an average length of 122 days in 1936 and 108 days in 1937.
[2] Mortality from 160 to 500 days of age.

For two of these lots, comprising pullets hatched in 1936 and 1937 from the dark and light strains, subsequent mortality was recorded from 160 days of age to January 23 of the following year.   In 250 unselected control pullets of 1937, mortality was recorded from 160 days to 500 days of age.   The figures, given in table 5, show that mortality differed slightly in the dark, medium, and light chicks, probably by chance and because of small numbers in some classes, but was not consistently higher in any one color class.   In the largest lot, the 1937 controls, mortality from housing time to 500 days of age was 4 percent less in 65 pullets originally having light down than in 81 birds that had the darkest down when hatched.   Moreover, considering only the pullets in this lot that died after 160 days of age, the mean ages at death for birds of dark, medium, and light downs were respectively 368, 387, and 390 days.   These differences in favor of the light-down pullets are not significant, but they do show that viability in such birds was equally as good as in those that originally had dark down.

CAPACITY FOR EGG PRODUCTION

TABLE 6.-- *Independence of down color and egg production in White Leghorn pullets, 1936 and 1937*

Year	Period of test, from first egg to-	Pullet egg production for indicated chick down color					
		Dark		Medium		Light	
		Pullets	Mean eggs	Pullets	Mean eggs	Pullets	Mean eggs
		Num- ber	Num- ber	Num- ber	Num- ber	Num- ber	Num- ber
1936	Feb 1, 1937	23	38	54	33	23	30
1937	Jan 23, 1938	21	42	41	58	26	55
1937, controls	500 days of age	47	162	56	160	40	166

The relation of down color to productivity was studied in the same three populations as were used to study viability.   The results, given in table 6, show no relation whatever between ability to lay eggs and color of down.   By chance, the most productive class had dark down in one lot, medium-color down in another, and light down in the third. In the longest and most significant test, that of the 1937 controls, pullets that originally had light down laid four eggs per bird more than those that had dark down as chicks.   The difference is quite insignificant but this test does show that the light chicks are not inferior to the dark ones.

IS THE YELLOW COLOR RELATED TO RIBOFLAVIN?

Because the light and dark strains of White Leghorns were differentiated while selecting for high and low requirements of riboflavin, which has a yellow color, it was desirable to determine to what extent differences in content of riboflavin were associated with differences in color of the down.   The yellow pigment in the down of White Leghorn chicks is not soluble in petroleum ether, or in chloroform.   It is partially dissolved, but not completely so, by digestion for 1 to 2 hours in a mixture of 25 percent normal sulfuric acid and 75 percent acetone. The extracts used in this study were obtained by this method.

Assays of the riboflavin content of these extracts were made in two ways. Photometric analyses of extracts from down collected in 1937 showed that the dark down contained the most riboflavin and the light down the least. Other samples taken in 1940 were kindly assayed by Dr. A. Z. Hodson according to the improved method of Hodson and Norris (4). The average content of riboflavin in two series of dark, medium, and light downs was respectively 3.9, 2.5, and 1.8 micrograms per gram of down. Since these are much smaller amounts of riboflavin than are found in red muscle or in glandular tissues, the concentration of that substance in the down is comparatively low.

It seems unlikely, therefore, that the differences in down color are related to riboflavin. The method used for its extraction is considered to remove most of the riboflavin, but even after its extraction the differences between the dark, medium, and light downs were practically as conspicuous as before extraction, despite marked coloration of the extract from the dark down. Furthermore, when the extracts were treated with a reducing solution of sodium hydrosulfite and sodium bicarbonate, the brownish pigment in them was not reduced to a colorless form as riboflavin usually is when so treated. For all these reasons, it seems improbable that differences in color of the down were dependent upon the presence of different amounts of riboflavin.

HEAD SPOTS

RELATION OF HEAD SPOTS TO COLOR OF DOWN

In contrast to chicks of most other breeds, White Leghorns are conspicuously lacking in patterns or stripes in the down. The nearest semblance to a pattern is a dark, brownish spot occurring on the back of the head. Some of these spots are quite large and conspicuous, others barely visible. Difference in size of the spot depends, not upon intensity of the color, but upon the number of long strands of pigmented down. Some of these include only two or three dark-colored strands and are barely evident. These head spots in White Leghorns are quite different from those of Rhode Island Reds, where a black or brown spot contrasts sharply with the lighter-colored, adjoining down.

TABLE 7.—*Distribution of head spots among White Leghorn chicks having down of dark, medium, or light color*

Population	Proportion of chicks with head spots in groups having indicated down color							
	Dark		Medium		Light		3 down-color classes combined	
	Total	With head spots	Total	With head spots	Total	With head spots	Total	With head spots
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
Dark strain, 3 years	209	99	20	100			229	99
Light strain, 3 years	51	76	521	26	196	51	768	36
Controls, 1937	229	96	493	77	105	27	827	76
F₂ generation from cross light × dark	68	91	95	74	18	39	181	77

The distribution of head spots in relation to color of down is shown in table 7. Head spots occurred in nearly every chick of the dark strain, but only in 36 percent of those in the light strain. The difference results in part from the fact that head spots are difficult to recognize on lightly colored chicks. However, because even in the dark chicks of the light strain the proportion showing head spots was lower (76 percent) than in dark chicks of the other populations, it seems probable that the genes causing light down may also tend to eliminate head spots. In the F_2 generation, the distribution of head spots was practically the same as in the unselected control population of 1937, both having head spots in about the same proportion of each color class and in about three-quarters of all chicks.

RELATION OF HEAD SPOTS TO SEX

In the control population, the light strain, and the F_2 generation, males comprised 71 percent of the dark chicks and females 61 percent of the light ones (table 3). Since head spots are found in over 90 percent of unselected dark chicks, but in less than 40 percent of the light ones (table 7), it follows that if all chicks in any population are considered, there must automatically be a preponderance of males among those with head spots, and of females among those without them. However, there is evidence that, apart from this spurious relationship, and regardless of down color, head spots are more frequent in males than in females. In the light strain and the unselected controls (table 7), there were 1,014 chicks of known sex classified as having down of medium color. The sex ratio in these— 514 males : 500 females—was normal. In this population, the distribution of sexes and head spots was as follows:

	Males	Females
Chicks with head spots	288	225
Chicks without head spots	226	275

Application of the chi square test to this distribution yields a value for χ^2 of 12.34 and for P of <0.001. Since the probability of such a distribution occurring by chance is less than 1 in 1,000, it is apparent that among chicks bearing head spots the proportion of males is significantly greater than among chicks lacking them.

This contrasts with the situation in Rhode Island Red chicks where, as Byerly and Quinn (1) and Hays (3) have shown, over 82 percent of the chicks with black head spots are females. In that breed, however, the spot is quite different in color, size, and shape from those found in White Leghorns.

DISCUSSION

Since the introduction by Punnett and Pease (9) of the Cambar, a breed of fowls in which the sexes of the chicks are recognizable at hatching because the females are darker than the males, the colors and markings of the down feathers of chicks have been studied by several investigators. Hagedoorn (2), Lamoreux (7), Jaap (5), and Punnett (8) have established new "autosexing" breeds in which, as in the Cambars, identification of sex at hatching is facilitated by a light ground color or pattern in the down which accentuates the normal difference between males homozygous for barring and females hemizygous for that sex-linked character. Even in pure Barred Plymouth

Rocks there are differences in color between males and females. Jerome (6) was able to identify sex in that breed with an accuracy of 98 percent by considering differences in the head spot, the color of down, and the color of shanks and feet.

In White Leghorns, as in all these autosexing breeds and in Barred Rocks, the males are homozygous for barring and the females are hemizygous, but these conditions apparently do not cause corresponding differences in color. In fact, since males predominate among dark chicks and females tend to have light down (table 3), what difference does occur is exactly the opposite of that in Barred Rocks and in the autosexing Cambars, Barnevelders, Ancobars, Oklabars, and Legbars, where the males are light and the females dark. Apparently any effect of the barring gene is as effectively masked by dominant white in chicks as it is in adult White Leghorns. Identification of sex by color of the down is impossible in White Leghorns, except for the generalization that the darkest chicks are more likely to be males and the lightest ones to be females.

SUMMARY

From a stock of White Leghorns there were differentiated two strains, in one of which about 83 to 94 percent of the chicks had dark down, while in the other only 3 to 12.5 percent had dark down, the remainder grading as medium or light in color. Because this sharp difference between the strains was established in 2 years without deliberate selection for color, and was subsequently easily maintained, it is considered that only a few genes caused the difference between the dark and light strains.

In F_1 generations from reciprocal crosses between these two strains, the proportion of dark chicks was intermediate between those in the parent strains. However, among the F_1 progeny from dark dams there was a higher proportion of dark chicks (63.6 percent) than in F_1 chicks from light dams (24.6 percent).

It was shown that sex-linked genes were not primarily responsible for differences in down color. The tendency for the F_1 progeny from reciprocal crosses to resemble the dam's strain more than that of the sire is attributed to some kind of maternal influence, the basis for which is as yet unknown.

In an unselected control population, the proportions of dark, medium, and light chicks were 28, 59, and 13 percent respectively. In chicks of medium color, the proportions of males and females were about equal. Of the dark chicks, 71 percent were males; and of the light ones, 61 percent were females.

Color of down was shown to be quite unrelated to size at hatching, early growth, age at sexual maturity, viability, and ability to lay eggs. In weight at first egg, the darker chicks were consistently the heaviest, and the paler chicks the lightest, but it is not clear that these associations are significant.

The amount of riboflavin was highest in dark down, and lowest in light down, but it is considered that down color is not determined by that substance.

Dark spots in the occipital region of the head occurred in 99 percent of chicks in the dark strain, 36 percent of those in the light strain, and 76 percent of unselected control chicks. In all strains they were

most frequent in dark chicks and least so in light ones. They were more frequent in males than in females.

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SIMULTANEOUS MEASUREMENT OF CARBON DIOXIDE AND ORGANIC VOLATILES IN THE INTERNAL ATMOSPHERE OF FRUITS AND VEGETABLES [1]

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INTRODUCTION

Maturity, handling, storage, and ripening induce changes in the metabolism of fruits and vegetables, which are reflected in the composition of their internal atmospheres. Quantitative analysis of such atmospheres should be of value in problems connected with gas storage, waxing, storage scald, core break-down, fungus decay, staleness, and the development of off-flavors.

The organic volatiles in such atmospheres contain certain odorous constituents, such as the amyl esters of formic, acetic, caproic, and caprylic acids (*12*),[2] together with acetaldehyde (*8, 10*), ethyl alcohol (*6*), ethylene (*9*), and essential oils. The common inert atmospheric gases are also present. As carbon dioxide is an end product of respiration, changes in the concentration of this gas assume special importance.

In a previous publication (*7*) Gerhardt and Ezell presented a method of estimating the organic volatiles emanating from stored fruit. The present paper describes a method of measuring simultaneously the total oxidizable volatiles and the carbon dioxide present in the internal atmosphere of fruits and vegetables. The method involves the refluxing of the plant tissue in an aeration stream of carbon dioxide-free air, the absorption of the organic volatiles in concentrated sulfuric acid and of the carbon dioxide in 0.1 N barium hydroxide, and the oxidation of the volatiles with 0.1 N ceric sulfate. The procedure permits the use of inexpensive laboratory equipment and lends itself to routine analysis with sufficient accuracy for most purposes.

REVIEW OF METHODS FOR MEASURING INTERNAL ATMOSPHERE IN FRUITS

CARBON DIOXIDE

Most of the methods for measuring the carbon dioxide content of the internal atmosphere in plant products have been fundamentally similar in principle, in that segments of tissue were surrounded with mercury and, after subjection to a vacuum, the expelled gases were collected, measured, and analyzed. Magness (*11*), using this principle, devised an extraction chamber and developed a technique of analysis employing the Bonnier-Mangin gas apparatus. Later Culpepper and coworkers (*5*), and more recently Brooks (*1*), employed

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enlarged extraction chambers and obtained sufficient quantities of gas to permit the use of the Orsat type of apparatus for analysis.

Although these methods have been used extensively and perhaps are suitable when comparing fruits of one kind, they may not give a true picture of the internal atmosphere. The total carbon dioxide in the tissue undoubtedly is present in different forms; namely, (1) as gaseous carbon dioxide in the intercellular air spaces and (2) as carbon dioxide in solution in the cell sap. Since the concentration of the dissolved gas in solution is directly proportional to the concentration in the free space above the liquid (Henry's law), application of a partial vacuum would immediately upset the equilibrium of such a system. Both Magness (*11*) and Brooks (*1*) recognized this fact in interpreting the limitations to be placed on the vacuum method as a measure of the total carbon dioxide in the internal atmosphere.

Willaman and Brown (*16*) studied the relationship between dissolved carbon dioxide and respiration in apple twigs, in which the total internal carbon dioxide was measured. Their method consisted essentially in submersion of the sample in boiling 95 percent ethyl alcohol in a closed container and removal of the carbon dioxide under reduced pressure by the use of CO_2-free air. The expelled carbon dioxide was entrapped in a standard alkali solution. Claypool (*4*) used a modification of this principle wherein the total internal carbon dioxide was measured by boiling the tissue in 200 cc. of distilled water for 15 minutes in a 1-liter Erlenmeyer flask. The liberated gases were swept out of the apparatus with CO_2-free air. and the carbon dioxide was absorbed in half-normal sodium hydroxide.

Wardlaw and Leonard (*15*) used a simplified modification of the method of Willaman and Brown (*16*) for measuring the total internal carbon dioxide in the banana. Their paper included an excellent diagrammatic description of the apparatus and its manipulation. Distillation flasks, condensers, and traps were fitted with ground-glass joints. The fruit tissue was immersed in boiling alcohol and aerated with CO_2-free air, and the liberated carbon dioxide was entrapped in Pettenkofer tubes carrying barium hydroxide. The variation of replicate samples was approximately 6 percent; loss of carbon dioxide, due to sampling, was of similar magnitude. Acidulation of the alcohol for extraction was found unnecessary, as any carbon dioxide present in combined form was liberated as a result of boiling with the acids already present in the tissue. Asymptotic curves, representative of carbon dioxide liberation, were obtained during reflux aeration of the normal alcoholic blank, from the alcohol charged with carbon dioxide, and also from green and ripening fruit tissue.

ORGANIC VOLATILES

Reference should be made to a recent publication by Gerhardt and Ezell (*7*) for a review of methods of measuring certain odorous constituents emanating from fruits and vegetables. Obviously, if these substances are liberated from the surface of intact fruits, they must exist in larger amounts in the internal atmosphere and cell contents of such fruits. Steam distillation of the tissue has been used as a general procedure for the removal of these volatiles.

Acetaldehyde (*6, 8, 10*) in the steam distillate has usually been trapped as the sodium bisulfite addition compound and determined

by iodometric means, whereas ethyl alcohol (6) has been oxidized to acetic acid with a sulfuric acid-potassium dichromate mixture. Christensen and coworkers (3) developed a new apparatus for the extraction of internal gases from plant tissue in connection with a bromination micromethod for the accurate determination of ethylene within a range of 0.001 to 0.06 cc. in an air sample of 35 to 40 cc.

Concentration of the steam distillates of the pulp and peelings of McIntosh apples by Power and Chesnut (12) produced pale-yellow viscous essential oils of pleasant applelike odor. These substances were present to a greater degree (0.0022 percent) in the parings than in the pulp (0.0012 percent). Hydrolysis of the esters in the distillates produced amyl alcohol and substances of roselike fragrance suggestive of geraniol. Oxidation of these alcohols led to the formation of valeric acid, acetone, and levulinic acid. The presence of the last two oxidation products, together with the purple coloration with Schiff's reagent, served to identify this volatile of roselike odor as the aliphatic terpene, geraniol.

EXPERIMENTAL PROCEDURE

PURPOSE AND BASIC PRINCIPALS OF PROPOSED METHOD

In connection with certain previous studies on the physiology of storage scald of apples, a method was devised for measuring the total volatile emanation from stored fruit (7). The rate of emanation of these volatiles from fruit in cold storage was often very low. It was recognized that, if these components could be measured as a group in the internal atmosphere of the fruit itself, their assay might be of greater importance to the solution of the problem.

The present method was evolved by combining a modification of the extraction technique of Wardlaw and Leonard (15) for the removal of total carbon dioxide from internal atmospheres, with the procedure described by Gerhardt and Ezell (7) for the absorption of volatile emanations from stored fruit. Briefly, it consists of refluxing the tissue in boiling distilled water; aerating with CO_2-free air; and passing the liberated gases through dual scrubbers, the first one containing concentrated sulfuric acid for the absorption of organic volatiles and the second containing 0.1 N barium hydroxide for the removal of carbon dioxide. Carbon dioxide and volatiles in the internal atmosphere of plant tissue are thereby measured simultaneously from the same sample in one operation.

DESCRIPTION OF APPARATUS

Figures 1 and 2 show the main features of the apparatus. The digestion equipment (fig. 1) consists essentially of the following parts: A 1-liter round bottom distilling flask with interchangeable ground-glass gas inlet tube, a soda lime trap and a Drechsel gas washing bottle carrying concentrated sulfuric acid, and a 10-inch Allihn reflux condenser bearing a Kjeldahl distillation trap and glass tubing for connection with the absorption towers in figure 2.

The essential features of the absorption and aeration equipment in figure 2 consist of the Truog absorption towers and the flowmeter. The towers of heavy glass tubing contain glass beads and are stoppered into 250-cc. side-arm extraction flasks. The first tower attached

to the extraction apparatus is 10 mm. in diameter, uses 2- to 3-mm. glass beads, and contains concentrated sulfuric acid for the removal of volatiles. The second tower is 20 mm. in diameter, uses 5- to 6-mm. glass beads, and contains 0.1 N barium hydroxide for the removal of carbon dioxide. The flowmeter is calibrated for 20 liters
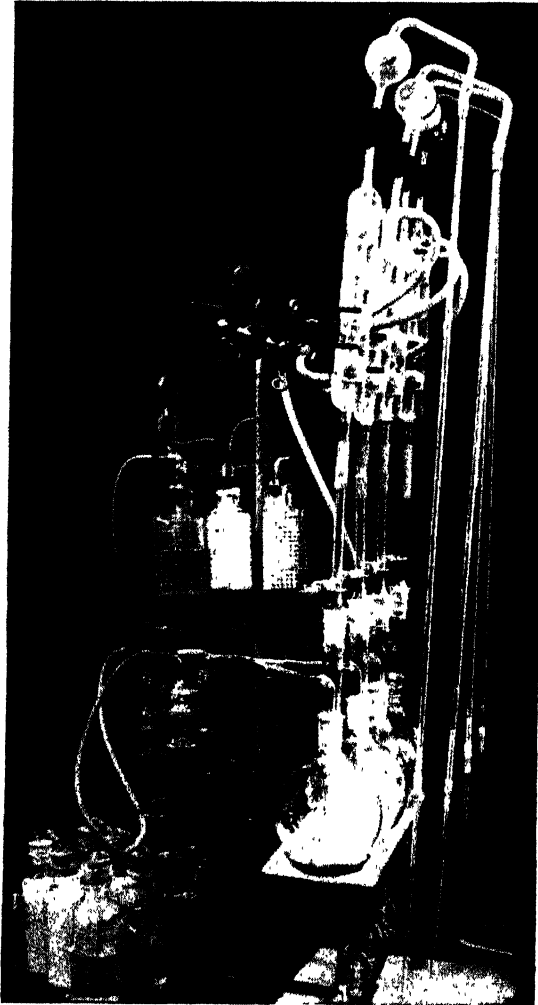
FIGURE 1.—Digestion and reflux apparatus for extraction of carbon dioxide and volatiles in the internal atmosphere of plant tissues.

of air per hour and connects the second tower with the source of aspiration through the surge chamber and mercury trap.

STANDARDIZATION OF TECHNIQUE

ABSORBENTS, OXIDATION, AND TITRATION OF ABSORBATES

In previous work (7), concentrated sulfuric acid (specific gravity, 1.84) was employed to absorb the volatile emanations from fruit.

growth shaded those receiving the other treatments. The data, however, may be considered fairly representative of what may be expected of 50° storage. In a previous season [8] 7- to 9-inch Creole bulbs produced 8 or 9 blooms in lots planted at once, 3 or 4 flowers after 5 weeks at 50°, and 4 or 5 blooms after 10 weeks' storage at 32°. In the trial under discussion, 5 bulbs in one block of the lot dug August 16 and planted at once produced 12 flowers per plant, showing a capacity to bloom equal to that of Creole bulbs forced in previous years.

No satisfactory explanation can be offered at this time for some significant differences found in size of flowers.

Number of leaves is markedly reduced by preplanting cool storage. This evidently reflects the earlier differentiation of flowers in lilies from cool storage. Flower buds are laid down terminally on the axis and prevent differentiation of additional leaves. Bulbs from 50° F. storage produced only 49 to 59 leaves before flowering, but lots not stored developed 141 to 145 leaves. Plants from 32° storage also showed a sharp reduction in number of leaves, and as such plants were very little shorter than nonstored plants they had conspicuously longer internodes. Following combination-storage treatments the number of leaves per plant was lower, or the change from vegetative to reproductive development was earlier than in the lots not cool-stored.

Results of variance analysis (table 1) show that storage treatment produced a very marked effect on time of emergence, time of flowering, plant height, number of leaves, and number of flowers, and a slight but significant effect on size of flowers. The effect of storage on number of flowers was less marked than in previous trials, apparently because competition suppressed some flowers in nonstored lots. Dates of digging also show a marked effect on time of emergence, time of flowering, and number of leaves because the bulbs proved more responsive to storage treatment at the earlier storage periods. Despite rather large and highly significant interaction between digging and storage treatments, the variance due to storage was significantly greater than that due to this interaction for the characteristics of time to emergence, time to flowering, plant height, number of flowers, and number of leaves.

EFFECT OF DELAYING COOL-STORAGE TREATMENTS ON FORCING PERFORMANCE OF CREOLE EASTER LILY BULBS

The Creole lily bulbs in the second experiment were handled similarly to those in the first experiment except that storage treatments were applied after some weeks in common storage in Louisiana rather than soon after harvesting.

Fifty bulbs were dug on the 1st and 15th day of each month from June 1 through August 1, and half the bulbs from each of these five dates of digging were stored at 50° F. for 6 weeks and half at 32° for 15 weeks. Lots 1 and 2, dug June 1, were placed in cool storage approximately 1 month after harvest. Lots 3 to 10 were all placed in storage August 15, approximately 2 to 8 weeks after their several dates of harvest. All lots were planted in the greenhouse immediately after removal from storage. The bulbs were smaller than those in the

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[8] See footnote 3.

TABLE 2.—Effect of delaying cool storage on forcing performance of Creole Easter lily bulbs

Lot No.	Storage Temperature	Storage Period	Digging date	Planting date	Mean flowering date	Period from planting to— Emergence	Period from planting to— Flowering	Plants flowering	Flowers per plant	Flower size (length×diameter)	Plant height
	°F.	Weeks	1939	1939	1939	Days	Days	Number	Number	Index number	Inches
1	50	6	June 1	Aug 16	Nov. 12	8.2±0.31	87.8±1.61	12	1.7±0.22	19.6±0.86	19.5±0.78
					1940						
2	32	15	do	Oct. 18	Mar. 16	35.6±2.57	150.3±3.85	15	2.4±.32	25.0±.42	30.8±1.31
3	50	6	June 15	Sept. 26	Feb. 9	17.1±.64	136.2±1.28	17	1.3±.11	23.5±.90	23.8±1.16
4	32	15	do	Nov. 20	Apr. 16	44.8±1.49	148.1±1.54	8	1.5±.19	26.3±.67	32.5±1.86
5	50	6	July 1	Sept 26	Feb. 15	19.2±.65	141.9±2.20	14	1.4±.23	25.3±.83	23.1±.98
6	32	15	do	Nov. 20	Apr. 7	31.9±1.40	139.5±2.01	17	2.1±.26	24.4±.58	28.4±1.06
7	50	6	July 15	Sept. 26	Feb. 20	19.2±.87	147.6±5.71	13	1.5±.22	23.5±.88	24.9±.85
8	32	15	do	Nov 20	Apr 7	39.3±2.03	138.8±2.35	8	2.3±.49	23.8±1.00	24.8±1.59
9	50	6	Aug. 1	Sept. 26	Feb. 11	17.4±.61	137.9±2.52	15	1.4±.13	22.3±1.13	24.0±.85
10	32	15	do	Nov 20	Apr 4	29.3±1.35	136.3±2.27	11	1.4±.28	22.6±.83	24.5±1.64

previous experiment and carried a high percentage of severe types of mosaic. They also were subjected to competition effects resulting from close spacing.

The performance of these bulbs, particularly with respect to number of flowers produced, was very unsatisfactory, but a few points of interest developed (table 2). Because the number of plants producing flowers varied from 8 to 17 out of a possible 25 in each lot, the only analysis attempted was the computation of means and standard errors for those flowering. These afford a rough measure of the differences required for significance between lots.

Lot 1, stored at 50° F. from July 5 to August 16, proved very responsive, as did similar lots in the trials just discussed, emerging in slightly more than a week and flowering in less than 3 months. The corresponding lot 2, stored at 32°, emerged in 36 days and bloomed after 150 days. The four lots, 3, 5, 7, and 9, harvested at different dates but all placed in storage at 50° at the same time (August 15), responded uniformly. These emerged in 17 to 19 days and flowered in 136 to 148 days, or from February 9 to February 20, showing differences of low significance or none. Evidently the season at which cool storage was applied determined the response. The date of digging appears to assume importance chiefly because it is necessary to dig early in order to store early. It is notable that storage at 50° at a comparatively late date (August 15 to September 26, lots 3, 5, 7, and 9) was much less effective in hastening flowering than similar storage earlier (July 5 to August 16, lot 1). In sharp contrast with the optimum response to 50° storage in early season, bulbs from 32° storage showed like performance regardless of the season of storage, all these lots emerging in 29 to 45 days and flowering in 136 to 150 days. The effect of early season storage (July 5 to October 18, lot 2) at 32° is thus not greatly different from that of later storage. There is, on the contrary, a general trend toward more prompt bloom in successively later plantings as noted in the previous experiment.

Plants from 50° F. storage treatments were comparatively short, ranging from approximately 19 to 25 inches. In the three earlier harvested groups, 2, 4, and 6, the plants from 32° storage were taller than those in the comparable 50° lots, but in the two later harvested groups, 8 and 10, they did not differ from the corresponding 50° lots, 7 and 9.

The flowers were few and small in this experiment, and data for both number and size showed variability not related to treatment. Disease content may have been responsible for part of this unsatisfactory performance. The effect of mosaic disease on forcing quality is discussed on page 231.

EFFECT OF PLACE OF PRODUCTION ON FORCING PERFORMANCE OF SEEDLING EASTER LILY BULBS

The bulbs in experiment 3 were nonselected mixtures of first-generation hybrids between commercial Easter lily varieties. They had grown for 2 or more years from seed, including 1 season in the production areas named. Bulbs were dug at Baton Rouge, La., Beltsville, Md., Raleigh, N. C., and Willard, N. C., on July 28, and at Los Angeles, Calif., on August 2, 1939. At Castle Hayne, N. C., they were dug in mid-June and held in common storage until late July. On August 11, 25 bulbs from each lot were stored at 32° F.

and 25 in common cellar storage at Beltsville. On October 20 all bulbs were removed from storage and planted in the greenhouse.

The planting design and the forcing practices were as described for the first experiment (p. 221). Again the number of flowers produced varied within lots, and there were many blind plants. In one block all five plants of the Castle Hayne sample from cellar storage were blind. Values for this group have been calculated according to Yates' [9] formula for missing plots. Much of the observed variability in number of flowers is attributed to crowding effects. The experimental data included in table 3 represent mean values from the five blocks in each instance. Variance analysis for the characteristics measured is also shown in table 3.

TABLE 3.—*Forcing performance of seedling Easter lily bulbs from six producing areas, stored at 32° F. or in a cellar from Aug. 11 to Oct. 20, when they were planted in the greenhouse*

EXPERIMENTAL DATA

Kind of storage and place of production	Mean bulb circumference	Mean flowering date	Period from planting to—Emergence	Period from planting to—Flowering	Flowers per plant	Flower size (length × diameter)	Plant height
	Inches	*1940*	*Days*	*Days*	*Number*	*Index number*	*Inches*
32° storage.							
Los Angeles, Calif	8 8	Mar. 21	33 7	153 2	8 5	28 6	39 5
Baton Rouge, La	8.9	Mar. 12	31.3	143.7	7 3	28 6	39 7
Beltsville, Md	5 8	Apr 30	44 4	192 9	5 7	24 9	28 4
Castle Hayne, N. C	5 3	Apr 5	33.1	168 5	1 5	31 6	34 1
Raleigh, N C	5 2	Mar. 24	29 9	155 7	2 2	31 1	33 2
Willard, N. C.	6.9	Apr 4	26 6	166.7	3 4	29 5	31 2
Mean	6 8	Mar 31	33 2	163 5	4 8	29 1	34 3
Cellar storage							
Los Angeles, Calif	9 7	May 11	20 5	204 2	8 1	26 9	49 7
Baton Rouge, La	10 0	Apr. 21	23 6	184 3	10 3	25 6	44 5
Beltsville, Md	5 3	May 14	42 2	207 4	3 3	24 9	31 9
Castle Hayne, N. C	4 5	May 13	35 3	205 8	1 7	26 6	35 0
Raleigh, N C	4 1	May 4	38 1	197 0	2 2	27 8	34 8
Willard, N C	6 2	May 13	20 6	206.5	3 3	26 9	34 7
Mean	6 6	May 8	30 1	200 9	4 8	26 5	38 5
General mean	6 72	Apr 19	31 62	182 16	4 79	27 75	36 40

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square [1] for—Days to emergence	Mean square [1] for—Days to flowering [2]	Mean square [1] for—Flowers per plant [2]	Mean square [1] for—Flower size [2]	Mean square [1] for—Plant height [2]
Blocks	4					
Places	5	504. 04**	1, 484 24**	95 34**	27. 12**	319. 68**
Storages	1	142. 30*	20, 988. 87**	02	101 14**	252. 56**
Places×storages	5	144 25**	370 19**	7 42*	7 00	27. 89
Blocks×places	20	23. 62	73 71	2 53	4. 39	18 04
Blocks×storages	4	20 07	91 25	4 61	3 83	25 62
Blocks×storages×places	20	27. 65	73. 97	1 80	4 66	21. 28
Pooled error	44	25. 13	75. 46	2. 40	4 46	20 18,
Total	59					

[1] * = Significant with respect to pooled variance indicated, ** = highly significant with respect to pooled variance indicated.

[2] 1 degree of freedom lost in a missing plot.

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Bulbs 7 to 9 inches in circumference were called for in the plan of the experiment, but only the Los Angeles and Baton Rouge samples permitted grading out 50 bulbs of approximately this size. Those from other sources were much smaller. Large bulbs are generally believed to flower somewhat earlier and to produce more flowers than smaller ones, but little is known of the relation of bulb size to other forcing characteristics.

Bulbs from the several production areas show differences in time of emergence. Those grown at Beltsville were slowest to emerge after both storage treatments. Lilies from Los Angeles and Baton Rouge came up more promptly after cellar storage than after 32° storage; those from Raleigh responded better after 32° storage; bulbs from Beltsville, Castle Hayne, and Willard showed no significant difference in effect of storage on emergence.

All bulbs stored at 32° F. flowered earlier than corresponding lots stored in the cellar. The advantage in favor of 32° storage is 37 to 41 days for bulbs from Baton Rouge, Castle Hayne, Raleigh, and Willard, 51 days for Los Angeles, and 14 days for Beltsville. Beltsville bulbs were clearly the least responsive to cool storing, and the Los Angeles bulbs possibly the most responsive. From cellar storage, 5 lots showed no significant departure from the mean requirement of 201 days to flower (May 8), the Baton Rouge sample alone showing distinct earliness (April 21). From 32° storage, 4 lots are grouped about the mean requirement of approximately 163 days (March 31), with Baton Rouge bulbs flowering earliest (March 12) and Beltsville bulbs latest (April 30).

With respect to height, lilies grown at Beltsville, Castle Hayne, Raleigh, and Willard are closely similar, but those from Baton Rouge and Los Angeles are taller. This difference is highly significant in plants from cellar storage and is lower but significant in plants from 32° storage. It is possible that this difference is related to the larger bulbs from Baton Rouge and Los Angeles. It is interesting to speculate whether there may be an additional tendency to tallness in lilies from the lower South as compared with a tendency to shortness in bulbs grown farther North.

No significant difference in number of flowers was found between storages, but between production areas there were several significant differences. In general, larger bulbs produced more blooms, as expected. Tall, early-flowering individuals also showed to advantage, since they suffered little from competition. Size of flower, which is not commonly shifted significantly by forcing methods, showed some tendency to higher values in plants that flowered early (March) and in plants that carried relatively few flowers.

Variance analysis (table 3) shows a highly significant effect associated with place of production on all characteristics measured. Between storage treatments are found differences in emergence, time of flowering, height, and size of flowers.

EFFECT OF MOSAIC DISEASE ON FORCING PERFORMANCE OF CREOLE EASTER LILY BULBS

The importance of mosaic in Easter lilies lies largely in its effect on forcing performance. No definite data are available on the degree of injury associated with the several mosaic types found in this species. Present field practice calls for roguing those types that seem destruc-

tive under field conditions. If data were available on the forcing performance of the several symptom types, roguing practices could be placed on a firmer basis.

The Creole lily bulbs included in this experiment represented three degrees of severity of the traditional lily mosaic or so-called necrotic fleck. The plants producing these bulbs were selected by D. L. Gill in the field in Louisiana during the growing season, when they were classified as (1) mild fleck, (2) medium fleck, and (3) severe fleck. The plants were labeled at this time, and after being dug they were stored for 10 weeks at 32°. Fifty bulbs of each class were then forced under conditions similar to those described for other experiments in this paper.

The data on performance are summarized in table 4. The standard errors afford a rough means of judging the significance of differences.

TABLE 4.—*Effect of three grades of mosaic on the forcing performance of Creole Easter lily bulbs dug on Aug. 1, 1939, in Louisiana, stored for 10 weeks at 32° F., and planted in the greenhouse Oct. 16, 1939*

[50 bulbs in each class]

Mosaic class	Mean flowering date	Period from planting to—		Flowers per plant	Flower size (length× diameter)	Plant height	Plants		
		Emergence	Flowering				Salable	Un-salable	Failing to bloom
	1940	*Days*	*Days*	*Number*	*Index number*	*Inches*	*Number*	*Number*	*Number*
Mild fleck	Mar. 19	50 9±1 06	155 4±1.72	4 6± 26	25. 2± 24	33 0±.57	31	18	1
Medium fleck	Mar. 17	50 7±1 48	153.3±2. 04	2 7± 22	24. 0± 41	29. 1±.74	12	32	6
Severe fleck	Mar. 26	56 7±1.49	161.7±1 99	1 9±.18	21 6± 59	22 1± 84	0	32	18

Since no disease-free lilies were included in this experiment, the mild-fleck class was chosen as the standard of comparison. Plants of severe-fleck history were slightly slower to emerge and to bloom, were much shorter, and bore fewer and smaller flowers. The differences in height and in number of flowers were particularly striking, both showing transitional values, in the medium-fleck class. Sixty-two percent of the mild-fleck and 24 percent of the medium-fleck class were judged salable according to standards of the Washington, D. C., market at Easter, 1940. This is in strong contrast with the severe-fleck class, in which no plants were classified as salable. The number of blind plants increased with the severity of the disease type, reaching 36 percent in the severe-fleck class. These results support the claim advanced earlier that severe mosaic affects height, number of flowers, and number of blind plants (see p. 227).

In view of the trend toward taller plants and to more and larger flowers in passing from severe fleck to mild fleck, one is tempted to infer that Easter lily seedlings are superior largely because they are virus-free. Commercial Easter lilies, including the Creole variety, are almost invariably affected with one or more viruses even though they show no symptoms.[10] The better commercial stocks carry low percentages of the necrotic fleck types included in this experiment (table 4) but are infected with mottling or latent viruses, which are

[10] BRIERLEY, PHILIP. PREVALENCE OF CUCUMBER AND TULIP VIRUSES IN LILIES. Phytopathology 30: 250-57, illus. 1940.

apparently-less injurious than mild fleck.   Data in table 1 from the commercial Creole lilies that were stored at 32° for 10 weeks and planted October 16 may reasonably be compared with the data in table 4.   These Creoles grew taller than the mild-fleck plants, but did not differ from the latter in number or in size of flowers.   The Baton Rouge seedlings from 32° storage (table 3) are also roughly comparable, in origin, bulb size, and treatment, with the mild-fleck Creoles of table 4 and with some of the Creoles of table 1.   These seedlings approximated such Creoles in height and considerably exceeded them in size and number of flowers.   The inference that virus-free seedlings force better than commercial stocks because they are disease-free is therefore tenable but not proved.

DISCUSSION

As shown in the first experiment, new-crop Creole Easter lilies may be flowered from November to January, depending upon the time of digging and upon the time of storing at 50° F.   This winter market is now favorable because it is customarily supplied with bulbs of the Giganteum variety stored from the previous season.   Although such bulbs deteriorate slowly in low-temperature storage (32° to 34°), their forcing capacity declines steadily with age.   When forced after 9 to 12 months' storage, bulbs 8 to 10 inches in circumference seldom average as many as three flowers.   The cool-stored domestic bulbs are thus competing with imported bulbs of lowest forcing performance.

Adaptability to forcing for the winter market is not peculiar to the Creole lily, although this is the only variety at present available in commercial quantities in the South.   Seedling Easter lilies grown at Charleston, S. C., have been flowered in December.   The suitability of a variety and of a production area for this early flower market is dependent largely on early maturity of the bulbs, for immature bulbs do not respond to 50° storage.

Little is known about the problem of maturity in Easter lily bulbs. It is clear that such bulbs when dug too early fail to respond to cool storage and develop so late after treatment that they represent a commercial loss.   In the 1939 season most of the Creole bulbs tested were sufficiently mature by June 16, and all but one proved satisfactory for cool storing by July 7.

SUMMARY AND CONCLUSIONS

Creole Easter lily bulbs were dug in Louisiana on six dates: June 16, June 26, July 7, July 19, August 1, and August 16, 1939.   As soon as the bulbs from each digging date reached Beltsville, Md., they were divided into six treatment groups: (1) Planted in the greenhouse at once; (2) stored at 50° F. for 5 weeks; (3) stored at 32° for 10 weeks; (4) stored at 80° for 3 weeks in open trays; (5) preliminarily stored at 80° for 3 weeks in open trays and then stored at 50° for 5 weeks; and (6) preliminarily stored at 80° for 3 weeks in open trays, and then stored at 32° for 10 weeks.   The 36 treatment-digging combinations, each represented by 25 bulbs, were randomized through 5 greenhouse benches.

Bulbs stored at 50° F. for 5 weeks flowered in 87 to 124 days after planting; the mean dates of bloom were October 23, November 13, December 2, December 12, 1939, and January 1 and January 28, 1940.

Bulbs planted without storage flowered from April 3 to 12, 1940, after 232 to 287 days in the greenhouse. Lilies from 32° storage bloomed from January 31 to March 14, 1940, after 135 to 152 days in the greenhouse. Bulbs receiving only 80° storage flowered from April 5 to 13, 1940. Those from the combination storage treatment at 80° and then 50° bloomed from December 23, 1939, to March 19, 1940, and those stored at 80° and then 32° bloomed from March 5 to April 14, 1940.

Bulbs from 50° F. storage emerged and flowered earlier, flowered at lower stature, and produced fewer leaves than those from other treatments including the bulbs planted on arrival.

A few plants, chiefly from bulbs dug in June 1939, failed to respond to the stimulus of cool storage, but flowered in April 1940, at the same time as those receiving no storage. It is suggested that these individuals were not mature enough to respond to storage.

However, there is no assurance that these bulbs will mature on the same date in different years. If maturity is retarded by unfavorable conditions in some years, early digging and cool storing for winter bloom may lead to serious losses. Furthermore, no data are available on the effect of region of production on time of maturity, but it seems reasonable to assume that bulbs will be ready to harvest in the Gulf States before such bulbs can safely be dug farther North. It is common knowledge that the tops of Easter lilies die down earlier in the South, and this is the chief index of maturity in practical use at present. Immaturity effects similar to those reported here were encountered by Thornton and Imle [11] in Easter lilies of the Erabu and Giganteum varieties grown at Yonkers, N. Y. Similar performance, probably attributable to digging when immature, is sometimes seen in commercial Easter lilies of these varieties reaching this country from Japan in September and October.

Creole lily bulbs are more responsive to the stimulus of 50° F. storage in July than they are in August and September, and the intensity of this stimulus diminishes as the season advances (table 1). The season of maximum response to the 5-week interval at 50° follows immediately the season of immature response, when flowering is not at all hastened by such cool storage. Creole lily bulbs dug in June 1939 included individuals responding and not responding. The sharp transition from no stimulus offers the physiologists a problem in interpretation. Moreover, it offers a practical hazard to growers who seek maximum earliness of flowering.

In regard to the practical problem of spreading the season of flowering from November to January, the results of the second experiment carry a suggestion that deserves examination and further trial. It appears from these data that as a preliminary to 50° F. storage common storage in Louisiana is not different in effect from leaving the bulbs in the ground. If such is the case, a single digging in the first week of July in a normal year followed by 50° storage after successive intervals in common storage should yield successive periods of flowering similar to those attained by the more cumbersome method of successive dates of digging.

In a second experiment with Creole lily bulbs the time of harvest was varied, but the storage dates (except for lots 1 and 2) were the same. Bulbs from 50° F. storage flowered at nearly the same time

regardless of the date of digging, and bulbs from 32° storage also were uniform in flowering season. The season when cool storage is applied is evidently of prime importance in determining the intensity of response; the time of digging is of importance only in that bulbs must be dug early to be treated. Storage at 50° was much less effective in hastening flowering when applied late in the season (August to September) than when applied in July.

Seedling Easter lily bulbs dug on approximately the same date in six different producing areas were forced after storage at 32° F. or in a cellar. From cellar storage Baton Rouge, La., bulbs were the earliest to bloom. From 32° storage Baton Rouge bulbs flowered earliest (March 12), and Beltsville, Md., bulbs latest (April 30), with those from Los Angeles, Calif., and from three points in North Carolina intermediate. Plants grown from bulbs produced at Beltsville, Md., Castle Hayne, N. C., Raleigh, N. C., and Willard, N. C., were similar in height, but plants from bulbs produced at Baton Rouge, La., and Los Angeles, Calif., were taller.

Creole lily bulbs from plants selected for three grades of severity of the necrotic fleck type of mosaic were compared. Bulbs from plants severely affected by fleck were slower to emerge and to bloom, much shorter, and bore fewer and smaller flowers than those from slightly affected plants. Thirty-six percent of the severe-fleck class failed to flower, and none of those that flowered was judged salable.

THE RELATIVE RESPIRATORY RATES AND HYGROSCOPIC EQUILIBRIA OF COMMON AND HULL-LESS OATS [1]

By P. E. RAMSTAD, *research assistant*, and W. F. GEDDES, *agricultural biochemist, Division of Agricultural Biochemistry, Minnesota Agricultural Experiment Station*

INTRODUCTION

The impression seems to be current among certain growers that hull-less oats (*Avena nuda* L.) are more susceptible to heating in storage than the ordinary varieties of oats (*A. sativa* L.). A series of respiration trials with Gopher oats and the hull-less variety South Dakota 165, grown at University Farm, Minn., in 1938, showed the respiratory rate of the former or ordinary variety to be appreciably higher than that of the hull-less oats at corresponding moisture levels. These results did not lend support to the idea that hull-less oats would heat more readily; on the other hand, they might be more hygroscopic than common oats and thereby exhibit a higher respiratory rate when stored at the same relative humidity. The experiments reported here were undertaken to recheck the respiratory rates of the two types of oats at similar moisture content and to determine their relative hygroscopicity.

MATERIALS AND METHODS

Unless otherwise noted, the varieties Gopher (common) and Nakota (hull-less) with test weights of 32 and 47 pounds per bushel, of high and approximately equal germinating power, grown at University Farm in 1939, were used. Respiratory rates were determined by the method used in previous studies at this laboratory. In brief, this method, which has recently been described in detail by Bailey,[2] consisted in incubating samples brought to moisture levels of approximately 11, 13, 15, and 17 percent at 37.8° C. (100° F.) for 4 days and determining the total carbon dioxide respired during this period by aspiration into standard barium hydroxide solution and back-titrating with standard hydrochloric acid solution. The moisture content of the samples was determined by a two-stage procedure, the first stage involving air-drying to hygroscopic equilibrium with the laboratory atmosphere, and the second, the determination of the residual moisture (after grinding in a Wiley laboratory mill to pass the 1-mm. sieve) by the vacuum-oven method of the Association of Official Agricultural Chemists.[3] In determining hygroscopicity, 3-gm. samples of the ground material were exposed in desiccators to atmospheres of known relative humidity, and the moisture content determined after constant weight had been attained. In adjusting atmos-

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pheres to the desired relative humidities over sulfuric acid solutions, the data of Wilson [4] were employed.

The studies of Gilman and Barron,[5] Bakke and Noecker,[6] and others have emphasized the importance of molds and other micro-organisms in increasing the respiratory activity of oats and other grains at high moisture content. It seemed probable that micro-organisms might develop more readily on hull-less than on common oats. Accordingly, two subsamples of each variety were prepared for each moisture level. The respiratory rates of one set of subsamples were determined 4 days after wetting and the rates of the other set after storage for 30 days at 37.8° C. (100° F.). By storing the samples in this manner, an opportunity was provided for the growth of molds and other micro-organisms on the samples that were of sufficiently high moisture content.

EXPERIMENTAL DATA

These respiration studies were carried out in duplicate, and the mean values are recorded in table 1. For equivalent sample treatments, the hull-less variety, Nakota, exhibited an appreciably lower rate of respiration than Gopher. Molds were evident in the samples of both varieties brought to the two highest moisture levels (15 and 17 percent) and held for 30 days at 37.8° C. These samples exhibited a higher respiratory rate at the end of 1 month than originally, but the comparative rates for Nakota and Gopher were essentially unchanged. There is thus no evidence of a differential behavior of these varieties in regard to the development of micro-organic activity so far as is disclosed by the level of respiration.

TABLE 1.—*The respiratory rates of Gopher (common) and Nakota (hull-less) oats determined at 37.8° C. (100° F.), 4 and 30 days after being brought to different levels of moisture*

Moisture content (percent)	CO_2 per 100 gm. of dry matter per 24 hours—				Moisture content (percent)	CO_2 per 100 gm of dry matter per 24 hours –			
	4 days after conditioning		30 days after conditioning			4 days after conditioning		30 days after conditioning	
	Gopher	Nakota	Gopher	Nakota		Gopher	Nakota	Gopher	Nakota
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*		*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
10.7		0.6		0 5	14.9	12.0		31.6	
10.8	0.9		0 5		15 2		4.5		22.0
12.8	2.2		8		16 8	37 2		41 2	
13.0		.9		.6	17.5		33 4		39.1

The moisture content of ground samples of the two varieties in equilibrium with atmospheres of different relative humidity, given in table 2, reveals that Gopher is slightly less hygroscopic than the hull-less variety, Nakota. That this difference may be ascribed entirely to a lower hygroscopicity of the hulls as contrasted with the groats

[4] WILSON, ROBERT E. HUMIDITY CONTROL BY MEANS OF SULFURIC ACID SOLUTIONS, WITH CRITICAL COMPILATION OF VAPOR PRESSURE DATA. Indus. and Engin. Chem 13: 326-331, illus. 1921.
[5] GILMAN, J. C., and BARRON, D. H. EFFECT OF MOLDS ON TEMPERATURE OF STORED GRAIN. Plant Physiol. 5: 565-573, illus. 1930.
[6] BAKKE, A. L., and NOECKER, N. L. THE RELATION OF MOISTURE TO RESPIRATION AND HEATING IN STORED OATS. Iowa Agr. Expt. Sta. Res. Bul. 165, pp. 317-336, illus. 1933.

Ethylene, however, is not appreciably absorbed in this reagent. Tropsch and Mattox (*14*) used sulfuric acid activated with nickel and silver sulfates to remove ethylene from gaseous hydrocarbons. This activated acid mixture, in the proportion of 1 part of concentrated sulfuric acid (specific gravity, 1.84) saturated with nickel sulfate at room temperature to 15 parts of concentrated sulfuric acid containing 0.6 percent of silver sulfate, was used in the present studies. Ten cubic centimeters of this acid mixture was employed
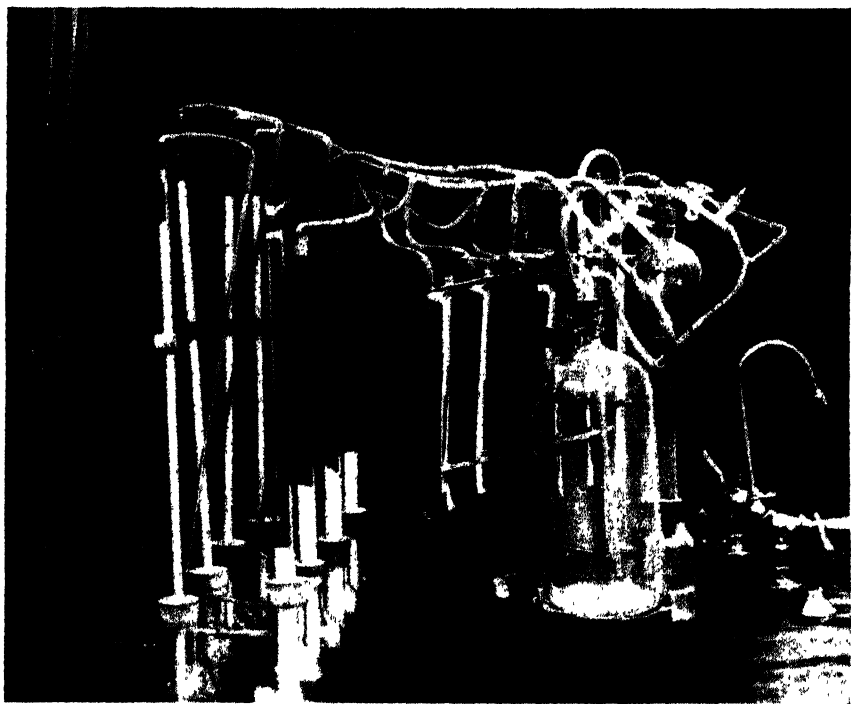
FIGURE 2.- Absorption and aeration equipment for measuring carbon dioxide and volatiles in the internal atmosphere of plant tissues; left to right, sulfuric acid tower for removal of volatiles, barium hydroxide tower for removal of carbon dioxide, flowmeter, surge chamber, and mercury trap.

for absorption of volatiles, and 25 to 50 cc. of 0.1 N barium hydroxide for the removal of carbon dioxide.

The oxidation of the volatiles with 0.1 N ceric sulfate dissolved in 0.5 M sulfuric acid was carried out as previously recommended by Gerhardt and Ezell (*7*). It entailed heating at 98° to 100° C. for 2 hours in a solution of at least 1 molar concentration in sulfuric acid. The amount of ceric sulfate reduced in the oxidation of the volatile material was determined by titration against 0.1 N ferrous ammonium sulfate, 0.005 M orthophenanthroline ferrous complex (*13*) being used as an indicator. Volatiles were expressed in terms of milligrams of ceric sulfate reduced per 100 gm. of tissue. Carbon dioxide, as milligrams per 100 gm. of tissue, was determined by titration of the reserve barium hydroxide in the absorption tower with 0.1 N hydrochloric acid, phenolphthalein being used as an indicator.

BLANK OR CHECK RUNS

Some carbon dioxide is initially present in the air within the extraction apparatus, and a certain amount of necessary contamination also occurs during dismantling of the absorption tower prior to titration of its contents. Slight reduction of ceric sulfate due to aeration and other manipulative practices should also be anticipated. These factors were studied by running a series of blank determinations. One hundred cubic centimeters of CO_2-free distilled water was added to each distillation flask. The absorption towers contained 10 cc. of activated sulfuric acid and 25 cc. of 0.1 N barium hydroxide. The boiling water in the distillation flasks was aerated at a rate of 20 liters per hour for 2 hours with CO_2-free air. At the close of the extraction period, any absorbates present were oxidized and titrated as described in the previous sections. The results are presented in table 1. These average corrections of 0.72 cc. and 0.25 cc. for carbon dioxide and volatiles, respectively, were used in all ensuing studies.

TABLE 1.—*Blank determinations by the proposed method for the analysis of internal atmospheres*

	For carbon dioxide			For organic volatiles	
Ba (OH)₂ (0.1 N) taken	HCl (0 1 N) back titer	Correction as indicated by Ba (OH)₂ (0.1 N) used (blank)	Ce (SO₄)₂ (0 1 N) taken	Fe (NH₄ SO₄)₂ (0 1 N) back titer	Correction as indicated by Ce (SO₄)₂ (0.1 N) reduced (blank)
Cc.	*Cc.*	*Cc.*	*Cc*	*Cc*	*Cc*
27 37	26 65	0. 72	25 10	24 80	0 30
27 37	26. 65	. 72	25. 10	24 85	. 25
27 37	26. 70	. 67	25 10	24 85	25
27 37	26 60	. 77	25 10	24. 90	20
Average		. 72	Average		25

EXTRACTION PERIOD FOR INTERNAL ATMOSPHERES

The influence of the length of the extraction period on the yield of volatiles and carbon dioxide was also determined. Two hundred grams of tissue of fresh Delicious apples was placed in each digestion flask along with 100 cc. of CO_2-free distilled water. The rest of the procedure was similar to that described for the determination of blanks. At intervals aeration was interrupted momentarily to insert fresh absorption towers into the hook-up. Data in table 2 show the amount of carbon dioxide and volatiles liberated at specific intervals over a period of 10 hours of extraction.

TABLE 2.—*Influence of length of extraction period on the analysis of carbon dioxide and organic volatiles in the internal atmosphere of tissue of Delicious apples*

Digestion period (hours)	Total CO₂ liberated per 100 gm. of fresh tissue	Amount of CO₂ liberated per additional period	Total Ce (SO₄)₂ reduced per 100 gm. of fresh tissue	Amount of Ce(SO₄)₂ reduced per additional period
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
1	4. 40		94. 2	
2	8 95	4 55	166. 0	71. 8
4	10 56	1. 61	192. 6	26. 6
6	12 15	1. 59	215. 7	23. 1
8	13. 60	1. 45	237. 3	21. 6
10	15 10	1. 50	256. 5	19. 2

There was a large increase in the liberation of these constituents between the first and second hours of extraction; thereafter, the additional amount removed in general grew progressively less with each 2-hour period of extraction. Undoubtedly the liberation of volatiles by steam distillation occurs at an asymptotic rate, although the limited data in figure 3 do not show this relationship. The sharp reduction in the quantity of volatiles removed after the second hour of extraction
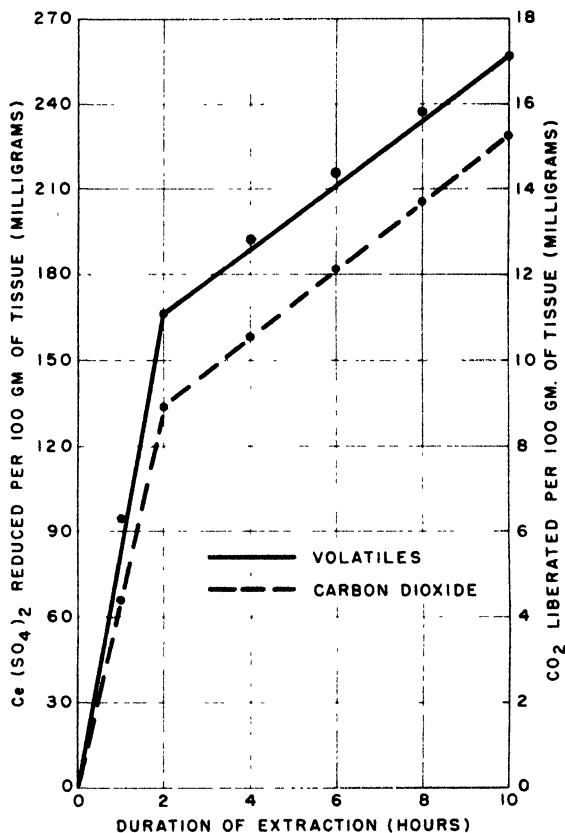
FIGURE 3.— Relationship between length of extraction and liberation of volatiles and carbon dioxide from Delicious apple tissue.

is substantiated by the experience of Wardlaw and Leonard (15) and also by that of Willaman and Brown (16) in their removal of carbon dioxide from plant tissues by aspiration in boiling alcohol. For all ensuing studies the period of extraction has been standardized to 2 hours of reflux and aeration.

INFLUENCE OF SAMPLING TECHNIQUE

One common lot of Delicious apples (sample A) and one of Winesap (sample B) previously stored at 31° F. were used in the study. Ten fruits of each variety were cored, quartered, and finely minced in a food chopper. A similar series of 10 fruits each were cored by passing a 1-inch cork borer from the calyx through to the stem of each fruit.

Samples of tissue were then removed for analysis by passing a ¾-inch cork borer through the greatest transverse diameter of the fruit. The plugs of fruit tissue were then transferred directly into the tared distillation flask of the apparatus. Two hundred grams of tissue per flask was used in each method of sampling. The remaining features of the technique were standardized as previously described.

The results, shown in table 3, indicate that considerable amounts of carbon dioxide were lost when the tissues were minced during sampling. The amount of volatile material, however, was not greatly influenced by either type of procedure. The losses in carbon dioxide were considerably greater than those reported by Wardlaw and Leonard (*15*), who permitted ground tissue to stand for different periods of time prior to immersion in the extraction apparatus. Evidently the major portion of the carbon dioxide loss occurs during grinding of the tissue and not during subsequent delay. Speed and adaptability recommend the cork-borer technique as the preferable means of sampling fleshy fruits and vegetables.

TABLE 3.— *Influence of sampling technique on the analysis of carbon dioxide and volatile material in the intercellular atmosphere of tissue of Delicious and Winesap apples*

Sampling procedure	CO_2 per 100 gm. of fresh tissue	Organic volatiles expressed as $Ce (SO_4)_2$ reduced per 100 gm. of fresh tissue
	Milligrams	*Milligrams*
Minced in food chopper.		
Delicious apples (sample A)	6.66	171 0
Winesap apples (sample B)	3 97	105 8
Cylinders of tissue removed by ¾-inch cork borer:		
Delicious apples (sample A)	10 04	168 0
Winesap apples (sample B)	7 56	108 7

EFFICIENCY OF METHOD

The accuracy of the proposed technique for the estimation of volatile constituents was tested by trial recovery of known amounts of acetaldehyde. Approximately 5 to 13 mg. of this volatile was added to the distillation flasks, and the analyses were carried out as recommended. Equivalent amounts of acetaldehyde were calculated from the milligrams of ceric sulfate reduced (based on a reduction-oxidation ratio of ceric sulfate to acetaldehyde of 51:1 as found by Gerhardt and Ezell (*7*)).

The results of this study are shown in table 4. They indicate that approximately 91 percent of the total acetaldehyde is recovered during the 2 hours of extraction as recommended in the proposed method. The rate of acetaldehyde removal from aqueous solution, like that of volatiles from Delicious apple tissue, is probably asymptotic in nature. Therefore, each increment of acetaldehyde recovered would require a progressively longer period of extraction, and beyond 91-percent recovery, further extraction would hardly be feasible for practical application.

A total recovery of 91 percent does not indicate a 9-percent variation between individual analyses of comparable samples. In such instances one should anticipate a variation of approximately 1 percent,

as shown in table 4. Analyses of such heterogeneous mixtures of volatiles as are found in the odorous constituents of plants hardly presuppose the accuracy of measurement possible with many inorganic compounds.

TABLE 4.--*Recovery of acetaldehyde by the proposed method for the analyses of internal atmospheres*

Acetaldehyde added (milligrams)	$Ce(SO_4)_2$ (0. 1 N) taken	$Ce(SO_4)_2$ (0. 1 N) reduced	$Ce(SO_4)_2$ reduced	Acetaldehyde	Recovered
	Cubic centimeters	Cubic centimeters	Milligrams	Milligrams	Percent
5 28	24. 9	7 4	245 8	4 81	91. 1
10 56	24 9	14 7	489 3	9 59	91 0
13.20	24 9	18.6	617. 9	12. 11	91.7
Average					91 3

RESUME OF DETAILS FOR PROPOSED METHOD

Sampling.--The cork-borer technique as previously described is recommended for sampling. The wall of the borer and the excised portion of peeling form a seal for the sample, which minimizes contamination with the atmosphere. The plugs of tissue are ejected directly into the tared 1-liter distillation flask containing 100 cc. of CO_2-free distilled water.

Extraction and absorption of internal gases.-- The extraction and absorption apparatus is assembled as previously described in the text and in figures 1 and 2. Usually 200 gm. of plant tissue is then refluxed and aspirated for 2 hours with CO_2-free air at the rate of 20-liters per hour. At the close of the extraction period the towers are dismantled and the glass beads are washed into the extraction flasks with CO_2-free distilled water (25 cc. for the acid tower and 50 to 75 cc. for the alkali tower).

Oxidation and titration of absorbates.--Twenty-five to 50 cc. of 0.1 N ceric sulfate solution (depending on the amount of volatiles expected) is added to the extraction flask containing the absorbed volatiles. Oxidation, titration technique, and expression of results for both volatiles and carbon dioxide follow the procedure previously described in this paper.

APPLICATIONS OF THE METHOD

Brooks, Cooley, and Fisher (*2*) suggested that the effectiveness of oiled wraps for control of common storage scald in apples may be due "to their power of absorbing esters or other similar products thrown off in gaseous form by the apple." By use of the proposed method, a test was made of the adsorptive capacity of plain and oiled paper wrappers for the odorous emanations from ripening fruit. Unwrapped Delicious apples, after 135 days' storage at 31° F., were removed to a ripening room at a temperature of 65°. One lot of fruit was wrapped in oiled paper (18.7 percent oil) and a second lot in plain paper wraps. After 10 days of ripening, the fruit wraps from both lots were removed and analyzed for their content of total volatiles. It was found that the oiled wraps contained 35.37 mg. of volatiles per wrap, whereas the plain paper carried only 3.82 mg. In other words, the oiled

paper took up approximately 10 times as much of the volatile emanations from apples as did the plain paper fruit wrap.

The influence of storage temperature on the accumulation of volatiles in the internal atmosphere of Winesap and Delicious apples was also studied. Data pertaining to this experiment are summarized in table 5. Total volatiles were calculated in terms of milligrams of both ceric sulfate reduced and of acetaldehyde. Furthermore, acetaldehyde was determined separately by steam distillation (6) of samples comparable to those used for the estimation of total volatiles. By this dual procedure, it was possible to show the proportion of the acetaldehyde to total volatiles and also how this relationship varied with the duration and temperature of storage.

TABLE 5.— *Influence of storage temperature on the accumulation of volatiles in the internal atmosphere of Winesap and Delicious apples*

[All lots of fruit wrapped in oiled paper; no scald present at the time of sampling]

| Variety | Storage temperature | Length of storage | Total volatiles per 100 gm. of fresh tissue expressed as— | | Acetaldehyde per 100 gm. of fresh tissue by actual analysis | Total volatiles due to acetaldehyde |
			$Ce(SO_4)_2$ reduced	Acetaldehyde		
	° F.	Days	Milligrams	Milligrams	Milligrams	Percent
Delicious	(¹)	0	69.7	1 3	0 16	12 3
Do	31	180	148 9	2.9	.58	20 0
Do	31	225	240 8	4 7	1. 14	24 2
Do	36	² 180	268 0	5 2	2 37	45 6
Do	36	² 225	513 5	10 0	4 97	49 7
Winesap	31	154	90.7			
Do	³ 30–40	154	132.2			
Do	36	154	260 7			

¹ At harvest.
² Abnormal, stale, acetaldehydic flavor.
³ Commercial storage.

The data in table 5 show that both total volatiles and acetaldehyde accumulate in the internal atmosphere of Delicious apples during storage. Furthermore, acetaldehyde evidently accumulates in greater amounts than other odorous constituents, since it forms an increasingly larger proportion of the total volatiles as the storage period is lengthened. After storage at 36° F. for 225 days, acetaldehyde accounts for approximately 50 percent of the total volatiles, and the flavor is definitely impaired. Previous unpublished studies have also shown a positive correlation between acetaldehyde accumulation and the development of stale off-flavors in the Delicious apple. In Winesap as in Delicious apples, there is also a greater accumulation of total volatiles during storage at 36° than at 31°.

Analyses of the internal atmosphere of a miscellaneous group of fruits and vegetables are shown in table 6. Ripening processes produced a much greater change in the internal atmosphere of the Bartlett pear than in that of the Golden Delicious apple. The increase in carbon dioxide during ripening amounted to approximately 65 percent in the pear and only 30 percent in the apple, whereas the organic volatile content increased more than tenfold in the former and only about 12 percent in the latter. A greater proportion of the total volatiles was composed of acetaldehyde in the Bartlett pear than

in the Golden Delicious apple, and, furthermore, the increase of acetaldehyde during ripening was approximately 80 times as great in the pear as in the apple tissue. After ripening at 65° F. for 7 days, the pear fruits were rather severely scalded, and the large increase in acetaldehyde during ripening may have been closely associated with the presence of this storage disorder, as previously reported by Harley and Fisher (*10*).

TABLE 6.—*Analyses of the internal atmosphere of different fruits and vegetables*

| Type of sample | CO_2 per 100 gm. of fresh tissue | Total organic volatiles per 100 gm. of fresh tissue expressed as— | | Acetaldehyde per 100 gm. of fresh tissue by actual analysis | Total volatiles due to acetaldehyde |
		Ce(SO_4)$_2$ reduced	Acetaldehyde		
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Percent*
Bartlett pear:					
Stored for 106 days at 31° F	13 00	107 4	2 1	0 31	14.8
Stored for 106 days at 31° F.; then ripened for 7 days at 65°	21 47	1,195 9	23 4	5.76	24 6
Golden Delicious apple					
Stored for 41 days at 31° F	6.66	126 4	2 4	.22	9.2
Stored for 41 days at 31° F.; then ripened for 7 days at 65°	8 69	140 8	2 7	29	10.7
Winesap apple					
Stored for 38 days at 31° F	9 81	101 4	1 9	.19	10 0
McIntosh apple					
Stored for 45 days at 31° F	8 69	98.2	1 9	21	11 0
Jonathan apples					
Soft scalded tissue	6 67	1,328 8	26.0	3 43	13 2
Sound tissue	7.79	297.4	5.8	92	15 9
Carrot [1]	41 80	531 5	10 4	00	0
Banana [1]	42 18	498 3	9 7	.00	.0
Sweetpotato [1]	48 50	307 2	6 0	00	.0
Potato [1]	41 39	149 4	2 9	.00	0

[1] Obtained from local fresh fruit market.

Differences in the composition of the internal atmosphere of normal and soft scalded tissue of Jonathan apples are also shown in table 6. The scalded areas contained slightly less carbon dioxide than normal tissue, whereas their total volatile content was approximately 5 times that of normal tissue. Evidently acetaldehyde accumulation cannot be the only factor in this large increase of total volatiles in the soft scalded areas, because, as shown in table 6, acetaldehyde comprises approximately a similar percentage (13 and 16) of the total volatiles in both types of tissue. It may be of more than passing interest to point out that both in the development of off-flavor in Delicious and in the formation of soft scald in Jonathan, total volatiles accumulated in large amounts. In the former case much of this increase was due to the accumulation of acetaldehyde (table 5), whereas in the latter, other types of volatiles were involved.

A higher carbon dioxide content and the absence of acetaldehyde are the main features that differentiate the internal atmosphere of the banana and certain vegetables from those of deciduous fruits (tables 5 and 6). One would scarcely anticipate that as there shown, the volatile constituents of the carrot were greater than those of the ripe banana, or even that those in the sweetpotato were present in larger amounts than those in the Golden Delicious or in the common Delicious apple.

SUMMARY

A method and an apparatus have been described for the simultaneous measurement of carbon dioxide and total volatiles in the internal atmosphere of fruits and vegetables. These analyses can be made simultaneously from the same sample of plant tissue.

The method involves (1) extraction of the internal atmospheres by refluxing and aspirating the plant tissue in boiling distilled water for 2 hours in an air stream of CO_2-free air at 20 liters per hour; (2) absorption of the components of the internal atmosphere in two gas scrubbers in series, the first bearing activated sulfuric acid for the removal of organic volatiles and the second bearing standard alkali for the removal of carbon dioxide; and (3) the oxidation of the organic absorbates with ceric sulfate.

Applications of the method include studies of (1) the adsorptive capacity of oiled fruit wraps for volatile emanations; (2) the influence of storage temperature, ripening processes, and soft scald on the composition of the internal atmosphere of fruits; and (3) comparative analyses of the internal atmosphere of certain vegetables and deciduous fruits.

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FURTHER STUDIES OF COOL STORAGE AND OTHER FACTORS AFFECTING THE FORCING PERFORMANCE OF EASTER LILY BULBS [1]

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INTRODUCTION

In previous experiments cool storage of Easter lily bulbs (*Lilium longiflorum* Thunb.) was most effective in hastening flowering when applied shortly after digging. Evidence from a small-scale trial with American-grown lilies during the 1938–39 season suggested that new-crop bulbs of a genetically early Easter lily variety, if dug early and subjected to optimum cool storage, might yield flowers for Christmas. Preliminary trials also suggested that bulbs dug before they were sufficiently mature could not be forced. Further experiments were therefore designed for the 1939–40 season to test the response to combined early digging and cool storage and to determine whether immaturity would assume practical importance in such a procedure. Preliminary trials to determine the effect of region of production on performance and the degree of damage associated with mosaic were also included. These experiments of the 1939–40 season are reported herein. Summaries of these experiments [2] and a report of the earlier tests [3] have already appeared.

EFFECT OF DATE OF DIGGING AND OF STORAGE TREATMENT ON TIME OF FLOWERING AND OTHER QUALITIES IN FORCED CREOLE EASTER LILIES

In Louisiana it has been customary to dig bulbs of the Creole variety in early August. Bulbs dug at this time in 1938 and stored shortly after digging at 50° F. for 5 weeks flowered by February 24, 1939. The primary purpose of the experiment described herein was to determine how best to produce satisfactory flowers for the winter season from new-crop bulbs. One hundred and fifty Creole lily bulbs were harvested in Louisiana on each of six dates: June 16, June 26, July 7, July 19, August 1, and August 16, 1939. Bulbs from each harvest were shipped to the United States Horticultural Station, Beltsville, Md., immediately after digging, and on arrival they were divided into 6 lots of 25 each, of which 5 lots were placed in controlled storage rooms and the sixth was planted in the greenhouse. Storage facilities were provided at the Arlington Experiment Farm, Arlington, Va., or at the Beltsville station. Temperatures in the storage rooms were controlled within a range of approximately ± 1° F.

Treatments included (1) storage of bulbs at 50° F. for 5 weeks; (2) storage at 32° for 10 weeks; (3) planting without storage; (4) preliminary storage at 80° for 3 weeks in open trays followed by storage at 50° for 5 weeks; (5) preliminary storage at 80° for 3 weeks in open trays followed by storage at 32° for 10 weeks; and (6) planting after preliminary storage (at 80° for 3 weeks in open trays) only. Each lot was planted in the greenhouse as soon as the storage period was completed. Inasmuch as no two lots were planted on the same date, each was subjected to a different growing environment. Such confounding of season of growth with treatment is not considered objectionable because bulbs subjected commercially to any of these treatments would encounter similar seasonal conditions. The 50° storage treatment is the most effective stimulus to early flowering yet found. Bulbs were stored for 5 weeks at this temperature because earlier trials [4] had shown this period to be effective and a 10-week period injurious. During the longer period excessive rooting and sprouting may take place in storage. The standards of comparison provided were (1) 32° storage, representing commercial practice, and (2) planting in the greenhouse at once without storage. The 32° storage was continued for 10 weeks because earlier trials had shown that bulbs so stored bloomed earlier than those stored for 5 weeks at this temperature. In previous years planting without storage produced the best growth of any practice yet tested [4] in terms of height, number of flowers, and number of leaves. This procedure may therefore be regarded as nearly ideal for growth of the Easter lily but not commercially practical because of the long time (up to 9 months) required for flowering. All three of these practices were applied to bulbs (1) immediately after arrival and (2) after preliminary storage at 80° for 3 weeks, to determine whether bulbs dug while immature would continue to mature in warm storage and therefore respond better to storage at 32° or 50°.

All bulbs were grown in 6-inch clay pots. The soil and manure were composted for a year and then were lightened by the addition of one-fourth muck and fertilized with bonemeal. The soil for the entire experiment was prepared at one time and thoroughly mixed to insure as much uniformity as possible. Whenever possible the forcing temperature was held at 60° to 65° F. During early fall and late spring this temperature range was of course far exceeded during sunny days.

The experiment (experiment 1) included 900 bulbs, representing 6 dates of digging, 6 storage treatments, and 5 replications of 5 bulbs each. The 25 bulbs of each lot were distributed 5 to each of 5 greenhouse benches (blocks), and the 36 lots were randomized in each block. Because of space limitations it was necessary to grow these lilies with adjacent pots against each other, and competition between plants strongly affected the number of flowers and induced some blindness.

The mean values for each lot of 25 plants (5 from each of 5 blocks) and the variance analysis for 6 factors recorded are given in table 1. The effects of treatment and dates of digging on time of flowering are shown in figures 1 and 2.

[4] See footnote 3.

TABLE 1.—*Effects of dates of digging and subsequent treatment on forcing behavior of Creole Easter lily bulbs produced in Louisiana*—Continued

ANALYSIS OF VARIANCE

Source of variation	Degrees of freedom	Mean square [2] for—					
		Days to emergence	Days to flowering	Flowers per plant	Flower size	Plant height	Leaves per plant
Blocks	4						
Diggings	5	1,035.05**	3,022.74**	1.57	2.61*	15.35*	795.39**
Storages	5	9,665.68**	109,523.60**	31.18**	2.83*	672.50**	45,170.40**
Diggings X storages	25	241.64**	1,289.50**	4.29*	2.62**	11.18**	190.02**
Blocks X diggings	20	15.32	40.27	3.35	.92	7.61	70.14
Blocks X storages	20	9.59	44.87	2.57	1.30	6.71	49.65
Blocks X diggings X storages	100	16.08	31.28	2.44	1.12	4.29	51.83
Pooled error	140	15.05	34.51	2.58	1.12	5.11	54.13
Total	179						

[2] *=Significant with respect to pooled variance indicated; ** =highly significant with respect to pooled variance indicated.

Emergence was accelerated strongly by storage at 50° F. and less strongly by storage at 32°. Bulbs planted without storage treatment emerged more promptly as the season advanced, suggesting a progressive maturity. A slight hastening in emergence associated with preliminary storage at 80° as compared with the lots not preliminarily stored indicates that the bulbs continued to mature at this temperature.

Preliminary storage at 80° followed by storage at 50° or at 32° resulted in slower emergence than the respective cool storages alone.

Two points of interest appear with respect to the number of plants flowering. Flowering of all 25 plants was most often attained in lots stored at 50° F., which treatment induced prompt emergence after a short (5 weeks) period in storage. The lots stored at 32°, although emerging in approximately 1 month after planting, were handicapped by the 10-week storage period. The lots not stored were slow to emerge, although they were the first after each date of harvest to be planted in the greenhouse. The lots stored at 80° and those receiving combination treatments including 80° suffered to some extent both from the delay in storage and from late emergence. The reduction in number of plants flowering, or increase in the number of blind plants per lot, seems primarily a competition effect resulting from too close spacing of plants. In general, plants emerging early flowered normally, whereas those planted late or emerging late suffered, presumably for light, from the competition of more advanced adjacent plants. In previous forcing trials [5] Creole lilies spaced to receive ample light developed few blind shoots after prolonged 32° storage or when planted without cool storage.

A further peculiarity in number of plants flowering is indicated by the numbers in parentheses in table 1, column 7, which refer to individuals failing to respond to cool storage. Such plants did not flower with the majority in the same lots but bloomed 3 or 4 months later. These are scored as not flowering since they failed to respond to cool storage, although they flowered later at the usual time for the variety. It seems that bulbs that are dug too early are not sufficiently mature

[4] See footnote 3.

to respond to treatment. This may be the reason that 20 of the 150 bulbs dug on June 16, 3 of those dug on June 26, and 1 of those dug on July 7 failed to respond to cool storage. Bulbs planted on arrival and those stored at 80° F. apparently completed their maturity during the 2 to 3 months that they required for emergence. Thornton [6] and Thornton and Imle [7] reported similar immaturity effects in Easter lily bulbs grown at Yonkers, N. Y.

In accelerating flowering, storage at 50° F. was strikingly effective (table 1), as in previous trials. This treatment was more effective when applied immediately after digging than after a 3-week period at 80°. Bulbs dug early proved much more responsive to 50° storage than those harvested later. Bulbs stored for 10 weeks at 32° flowered much more promptly than bulbs not cool-stored, but they were slower to flower than bulbs from 50° storage. A period of 3 weeks at 80° followed by 10 weeks at 32° was less effective in hastening flowering than 32° alone. The lots not subjected to cool storage bloomed during the first 2 weeks of April regardless of the time of planting (June 23 to August 22). Bulbs receiving only 80° storage bloomed during the same period in April. This suggests a normal flowering season for the Creole variety, which is altered only by strong stimuli, such as cool storage.

With regard to actual dates of flowering it may be seen from table 1 that the 50° F. storage applied to bulbs from the six successive dates of digging yielded a reasonably even spacing of bloom from October 23 to January 28. Similarly, 32° storage produced flowers at intervals from January 31 to March 14. Thus, digging at different dates might serve as a means of controlling time of bloom. Such a means, however, is impracticable and other methods of timing are required.

Lilies from 50° F. storage flowered on significantly shorter stems than those from other treatments. Plants in lots not cool-stored were tallest, but not significantly taller than those in lots receiving 80° storage only. Lilies from 32° storage and those from the combination storage treatments of 80° and 50° and 80° and 32° were intermediate in height between the lots stored at 50° and those planted on arrival. All Creole plants in this experiment grew taller than in similar experiments of previous years, those in the 50° lots averaging about 10 inches taller. Also, those in the 50° lot dug on August 16 flowered more than 2 weeks earlier than those receiving a comparable treatment in the previous year. Placing the pots on raised benches rather than on ground beds as in earlier trials may have been responsible for these differences.

The data for number of flowers are misleading. The random distribution of the 36 lots through the 5 blocks (benches) and the close spacing led to severe competition between plants. The effects of this competition appear chiefly as reduced numbers of flowers and as blind plants, as previously mentioned in the discussion of number of plants flowering. Reduction in number of flowers was notable in the lots planted at once because these were slow to emerge, and also in the 32° F. and 80° and 32° combination lots because these were delayed in planting by long periods in storage. The net effect is to show these other treatments at a disadvantage, since the lots from 50° storage emerged promptly, bloomed early, and their vigorous

[6] Thornton, Norwood C. Development of dormancy in lily bulbs. Boyce Thompson Inst. Contrib. 10: 318-388, illus. 1939.
[7] ——— and Imle, E. P. Why a dwarf Easter lily? Florists' Exch. 94 (15): 9, illus. 1940.

was shown by studying the hygroscopic behavior of these two fractions prepared from Gopher oats by hand-hulling without previous parching or heat-treating. The hygroscopicity of the groats, which comprised approximately 75 percent of the kernel, was virtually the same as that of the hull-less variety, Nakota. At 70 percent relative humidity the groats contained 13.8 percent and the hulls 12 percent moisture.

The lower hygroscopicity of the hulls appears to provide a partial explanation of the higher respiratory rate of Gopher as compared with Nakota oats. At corresponding moisture levels, the groats of Gopher actually have a higher moisture content than those of Nakota and hence would be expected to respire at a more rapid rate. To investigate this point the respiratory rates of groats and the oats from which they were prepared were determined at a series of moisture levels as previously outlined. The groats were made from a commercial sample of untreated high-grade oats by means of a "green-oat huller" with the machine adjusted to produce the minimum mechanical and heat damage. The results, shown in table 3, reveal that at the same total percentage moisture content, whole oats have a higher respiratory rate than the groats, thus confirming the deductions drawn from the lower hygroscopicity of the hulls.

TABLE 2. — *The hygroscopic moisture of ground samples of Gopher (common) and Nakota (hull-less) oats in atmospheres of different relative humidity at approximately 25° C.*

Relative humidity (percent)	Equilibrium moisture content of—		Relative humidity (percent)	Equilibrium moisture content of—	
	Gopher	Nakota		Gopher	Nakota
	Percent	*Percent*		*Percent*	*Percent*
35	9 0	9 7	68	13 1	13 7
50	10 7	11 4	81	16 9	17.2
60	11 6	12 3			

TABLE 3. — *The respiratory rates of oats and the groats prepared therefrom, determined at 37.8° C. (100° F.) 4 days after being brought to different levels of moisture*

Moisture content (percent)	CO_2 per 100 gm of dry matter per 24 hours for—		Moisture content (percent)	CO_2 per 100 gm of dry matter per 24 hours for—	
	Oats	Groats		Oats	Groats
12.6		0 5	13 9	1 7	
12 8	0.9		14 5		6 2
13 7		1 4	15 8	27.9	24 7

DISCUSSION

These studies, while limited in scope, confirm the findings of preliminary studies undertaken previously with Gopher oats and the hull-less variety South Dakota 165, regarding the higher respiratory rates of Gopher as compared with hull-less oats at the same total moisture content. The higher respiratory rates of common oats, as represented by Gopher, even under conditions which favor mold

development, arc the reverse of what would be expected on the basis of the impression held by growers that the hull-less varieties are more susceptible to heating in storage. Even when allowance is made for the higher hygroscopicity of Nakota, examination of the data presented here indicates that, when stored under the same conditions as regards atmospheric humidity, Gopher oats would still have a slightly higher respiratory rate.

It must be emphasized, however, that the respiratory rates have been compared on the basis of unit weight of dry matter per unit of time. Since the test weights of Nakota and Gopher oats were 47 and 32 pounds per bushel, respectively, there is approximately 50 percent greater weight per unit of volume in the case of hull-less oats. Thus, on a unit volume basis, the respiration of the hull-less oats would be greater than that of common oats if the air supply were unlimited. But there is also less air space per unit volume in the case of the hull-less oats, and under normal bin storage conditions the well known inhibitory effect of accumulated carbon dioxide on respiration would come into play sooner. These considerations make it appear quite possible that hull-less oats would decrease in viability more rapidly than common oats when stored in bins.

It would appear, then, that as far as danger from heating is concerned, hull-less oats could be safely stored at fully as high a moisture content as ordinary oats, but it would be necessary to investigate more thoroughly their susceptibility to micro-organisms before a definite conclusion can be reached. As far as storing oats for seed purposes is concerned, the present practice of preservation in bags stacked to permit free access of air should be continued until comparative data are available for the relative changes in germination capacity of oats stored in bags and bins over a period of time.

SUMMARY AND CONCLUSIONS

Respiration studies employing Gopher (common) and Nakota (hull-less) oats of high germinability and with test weights of 32 and 47 pounds per bushel, respectively, showed that, at the same total moisture content, the respiratory rate of Gopher per unit of dry matter was appreciably higher than that of Nakota, even under conditions which favored mold growth.

Gopher oats in equilibrium with atmospheres of different relative humidity were less hygroscopic (approximately 0.7 percent) than Nakota oats. This difference was shown to be due to the low hygroscopicity of oat hulls, which comprise about 25 percent of the kernel; at 70 percent humidity oat groats contained 13.8 percent moisture and oat hulls 12 percent moisture.

The higher respiratory rate of Gopher oats as compared with Nakota at the same total moisture content, is due in part to the low hygroscopicity of the hull and consequent higher moisture content of the respiring material, as shown by the fact that oat groats had a lower respiratory rate than the commercial oats from which they were prepared. At corresponding relative humidities, calculations indicate that Gopher oats have only a slightly higher respiratory rate than Nakota.

The slight differences in the size of bulbs supplied from the six dates of digging and also in the subdivision of these six lots into the six storage comparisons did not seem to have produced any important effects on the characters under study.
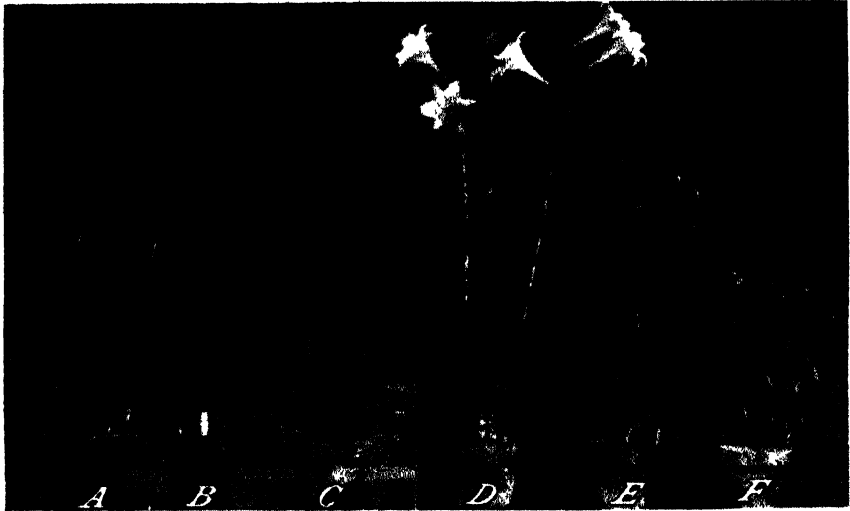
FIGURE 1.—Effect of storage treatment on time of bloom in Creole Easter lilies grown in 6-inch pots from bulbs harvested July 19, 1939: *A*, 80° F. for 3 weeks, then 50° for 5 weeks; *B*, 80° for 3 weeks, then 32° for 10 weeks; *C*, 80° for 3 weeks; *D*, 50° for 5 weeks; *E*, 32° for 10 weeks; *F*, planted on arrival. Photographed December 27, 1939.
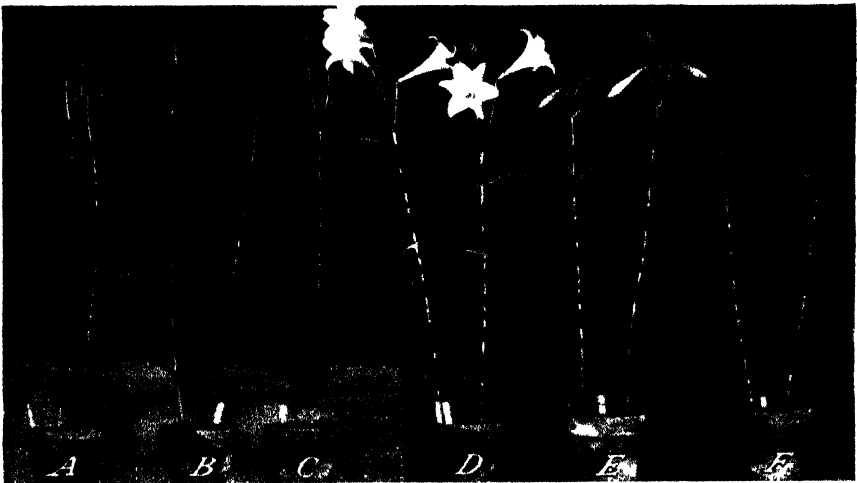
FIGURE 2.—Creole Easter lilies grown in 6-inch pots from bulbs harvested on six different dates and stored shortly after harvest for 5 weeks at 50° F.: *A*, Harvested June 16, 1939, flowered October 23; *B*, harvested June 26, flowered November 13; *C*, harvested July 7, flowered December 2; *D*, harvested July 19, flowered December 12; *E*, harvested August 1, 1939, flowered January 1, 1940; *F*, harvested August 16, 1939, flowered January 28, 1940 Photographed December 27, 1939.

TABLE 1.—*Effects of dates of digging and subsequent treatment on forcing behavior of Creole Easter lily bulbs produced in Louisiana*

EXPERIMENTAL DATA

Preliminary and storage treatment	Digging date	Planting date	Mean flower-ing date	Period from planting to— Emer-gence	Period from planting to— Flow-ering	Plants flower-ing [1]	Flow-ers per plant	Flower size (length × diam-eter)	Plant height	Leaves per plant
	1939	*1939*	*1940*	*Days*	*Days*	*Number*	*Number*	*Index number*	*Inches*	*Number*
Planted immediately	June 16	June 23	Apr. 5	83.4	287 2	22	5 7	25.0	43.6	145 3
	June 26	July 5	Apr. 3	79.2	273 4	21	4 9	25.1	42.0	143.2
	July 7	July 15	Apr. 10	72 8	269.7	20	4.5	25.7	43 0	143 2
	July 19	July 25	Apr. 12	61 5	261.9	23	5 0	24.2	39 8	144.3
	Aug. 1	Aug. 7	Apr. 10	51.2	246 6	23	6 2	24.9	43.3	143.7
	Aug. 16	Aug. 22	do	45.4	232 3	24	6 2	25 0	41 7	140 7
Mean				65 6	261 9	...	5 4	25.0	42.3	143 4
Stored immediately.			*1939*							
	June 16	July 28	Oct. 23	18 1	87 0	21(+4)	3.7	24 5	27.4	54 7
	June 26	Aug. 9	Nov. 13	14 4	95.5	24(+1)	3 9	25.5	27 6	51.8
5 weeks at 50° F	July 7	Aug. 19	Dec. 2	17 2	105.5	25	6 4	26 4	29 7	54 2
	July 19	Aug. 29	Dec. 12	17.3	104.7	25	5.2	26 5	27.8	49 0
			1940							
	Aug. 1	Sept 11	Jan. 1	13 2	112 1	25	5 1	25.8	31 2	55.1
	Aug 16	Sept. 26	Jan. 28	17 4	123 9	25	4 1	24 6	31 8	58 8
Mean				16 3	104.8	...	4.7	25 6	29 2	54.0
	June 16	Sept 1	Jan. 31	35.0	151.9	15(+9)	4 8	25 5	38 1	73 3
	June 26	Sept. 13	Feb. 8	35 1	147 9	23(+2)	4 0	25 3	37.4	68 0
10 weeks at 32° F	July 7	Sept. 23	Feb. 18	35 3	148 5	23	3 8	25 6	38.1	67 8
	July 19	Oct 3	Feb. 20	27 2	139 8	19	2 4	24 2	37 9	67 2
	Aug. 1	Oct. 16	Mar 2	33 6	137 9	23	4 3	24.8	39 9	60 6
	Aug. 16	Oct 31	Mar 14	32 8	134 7	23	3 9	25 4	38 4	56 6
Mean				33 2	143 4	...	3 9	25 1	38.3	65 6
Preliminarily stored 3 weeks at 80° F..	June 16	July 14	Apr. 7	72 1	268 1	22	4 0	25 0	41.8	135.5
	June 26	July 26	Apr. 13	62.8	261 8	19	5 6	25 5	42.3	139 4
Planted without subsequent storage.	July 7	Aug. 5	Apr. 8	57 8	247.1	24	5 0	26 3	43.3	144 2
	July 19	Aug 15	Apr 5	50 0	234 2	24	4 8	25 0	41 2	128 8
	Aug 1	Aug. 28	Apr. 9	48 7	224 7	20	3 8	25 1	40 6	126 2
	Aug. 16	Sept. 12	Apr. 5	43.4	206 2	20	3.4	25 6	39 6	110 6
Mean				55 8	240 3	...	4 5	25 4	41 5	130 8
			1939							
	June 16	Aug. 18	Dec. 23	32 4	127.3	20(+4)	5.1	27.7	34.9	70 6
			1940							
5 weeks at 50° F..	June 26	Aug. 30	Mar. 9	46.1	191.6	21	3.7	25 0	38 4	86 0
	July 7	Sept. 9	Mar. 4	45 7	177 2	19(+1)	2.2	25.2	36 6	76 2
	July 19	Sept. 19	Feb. 7	27.4	141 4	25	4.2	26 1	33. 4	65.7
	Aug. 1	Oct 2	Feb. 26	29.0	146.7	24	3.1	25 3	34 2	65.0
	Aug. 16	Oct. 17	Mar. 19	32 9	153.9	22	3 4	24 8	38 8	70.2
Mean				35.6	156 3	...	3.6	25.7	36.1	72.3
	June 16	Sept. 22	Mar. 5	52 1	165 2	22(+3)	3.5	25 6	40.4	69.8
	June 26	Oct. 4	Mar. 28	50 6	175.9	21	2.6	25.9	39.3	71.2
10 weeks at 32° F	July 7	Oct. 14	Apr. 3	58 2	171.7	15	1 0	26.5	39.6	66.8
	July 19	Oct 24	Apr. 1	53.8	160 4	19	2 1	25.7	38.8	60.0
	Aug. 1	Nov. 6	Mar 29	46 8	144.2	19	2.6	24.5	37.8	51.8
	Aug. 16	Nov. 21	Apr. 14	46.8	144 7	22	3.0	26 2	40.3	47.5
Mean				51 4	160 4	...	2.5	25.7	39.4	61.2
General mean				43.0	178.0	...	4.1	25.4	37.8	87.9

[1] 25 bulbs were planted in each lot; numbers in parentheses represent additional plants flowering far later than date given.

The data do not afford any basis for the assumption that hull-less oats would prove more susceptible to heating in storage, an assumption which has been more or less common among certain growers. Hull-less oats, however, have less interseed air space than common oats, and, under bulk storage, the carbon dioxide concentration in this space would tend to increase more rapidly. It is quite possible that these conditions would favor loss of viability in the bulk storage of hull-less oats at moisture levels which would provide a sufficiently high rate of respiration to result in the accumulation of carbon dioxide.

DIAMETER OF FIBER IN DIFFERENT STRAINS OF ACALA COTTON [1]

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INTRODUCTION

In the last few years the quality of cotton fiber has become increasingly important as a result mainly of a greater demand for better quality goods and the competition of both synthetic fibers and foreign growths. This has led to an increasing amount of research to determine the characters that go to make up quality in cotton fiber.

The main purpose of the investigation described in this paper was to determine what differences, if any, occur among related strains in respect to fiber diameter. Whether the method of swollen diameter is the best method for the breeder to use for fiber fineness, should he desire to know the fineness of his strains, requires further investigation. The diameter of fiber to be of great service would have to be used along with length, fiber weight per inch, strength, and other characters. There are several methods which could easily be adapted to any testing program for fineness of fiber (2, 4, 8, 15).[2]

LITERATURE REVIEW

Pierce and Lord (9) first suggested the use of swollen diameter of fiber as an estimate of the intrinsic fineness of a cotton.

Hutchinson and Govande (6) showed that fibers of different species of cotton from different parts of the world varied greatly in hair weight and in swollen diameter. In certain samples they found significant correlation coefficients between hair weight per inch and swollen diameter, which seemed to show that swollen-fiber diameter could be used as a measure of fineness. In Malvi samples (*Gossypium arboreum* var. *neglectum* forma *bengalensis*) they found no significant correlation between hair weight and swollen diameter, but they did find a significant correlation between spinning value (or highest standard warp counts) and swollen diameter, the coefficient being -0.580, $P=0.01$. Between spinning value and hair weight they also found a significant correlation, namely, -0.597, $P=0.01$.

Hutchinson and Govande (6, p. 43) stated:

Comparison of Pierce and Lord's (1934) [9] data for the relation between swollen hair diameter and hair-weight, with those available for Malvi cottons shows a much lower correlation in the latter than in the former. Environmental effects appear to have lowered the correlation between swollen hair diameter and hair-weight considerably, but not to have affected that between swollen hair diameter and spinning value to any great extent.
* * * the environmental variances are in all cases very small compared with the crop variety variances. Environmental correlations also are usually small, so that in general the plant breeder is safe in assuming that much the greater part of the differences, he observes, is of genetic origin.

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For plant breeding purposes, the labour involved in the determination of hair-weight is prohibitive. The demonstration by Pierce and Lord (1934) [9] of the close relationship between hair-weight and swollen hair diameter opened up a new method of determining fineness, and the data given above for hair-weight, spinning value and swollen hair diameter in closely related Malvi strains tends to confirm the value of the method for estimating fineness in the kind of material with which the plant breeder works, though further work is necessary. * * * The development of a method whereby selection for high ginning percentage could be carried on without risk of developing a coarse lint is one of the most pressing problems in cotton breeding, and the determination of swollen hair diameter provides a possible solution.

Koshal and Ahmad (7), who have developed a method for determining swollen diameters, found in standard Indian cottons significant correlation coefficients between swollen diameter and fiber weight per inch, $r=0.746$; swollen diameter and standard hair weight, $r=0.839$; swollen diameter and spinning value or highest standard warp counts, $r=-0.800$. They state (7, *pp. 11–12*):

The correlation between highest standard warp counts and swollen diameter is negative and significant, consequently this fiber property can be utilised for the identification of cottons having different spinning values, or may be coupled along with the other fiber properties for predicting the spinning value of a cotton. For this purpose, however, it would be necessary to carry out swollen diameter measurements on a large number of cottons for which the other necessary data is available.

The present report is composed of three parts: (1) Swollen-diameter measurements of each length as pulled from the fiber sorter on 6 strains, 2 of which were grown in 2 different localities in 1938; (2) swollen-diameter measurements on the 1⅛-inch fibers of 12 strains of the advanced-strain test at State College, N. Mex., grown in 1938, and of 16 strains of a similar test grown in 1940; and (3) measurements of ribbon width, thickness, and number of convolutions of the 1⅛-inch length fibers of 4 strains and the relationship of these 3 characters in each strain.

MATERIAL AND METHODS

Samples of cotton (*Gossypium hirsutum* L.) taken from each plot of the advanced strain test were used. To obtain the swollen-diameter measurements in experiment 1, a sample of 10 seeds was selected, 1 from the middle of 1 lock of each of ten bolls. This is the basic method of taking a sample to be placed in a fiber sorter for obtaining the distribution of different lengths of fibers by weight in a sample of cotton.

The lint on this sample of 10 seeds was combed out very carefully and placed in a Pressley hand sorter. Then a sample of each length was drawn from the sorter and placed crosswise on a microscopic slide, a cover glass was placed over it, and the fibers irrigated with an 18-percent sodium hydroxide solution according to the method of Koshal and Ahmad (7). After 10 minutes the slide was placed under the microscope with a Euscope attachment and 100 fibers were measured by the aid of a micrometer eyepiece. Koshal and Ahmad (7) found that to obtain an accuracy of about 4 percent of the mean diameter with odds of 99:1, tests of diameter must be made on 90 fibers. If two similar cottons are to be tested for accuracy, 200 fibers must be used. In experiment 1, the measurements were made on fibers of strains 1064, 1980, 1517, 2780, 2768, and Sh 5 grown at State College, and on strains 1064 and 1517 grown at Roswell, N. Mex.

In experiment 2 samples were prepared as described above and 100 fibers were measured from each plot of the advanced-strain test at State College in the seasons 1938 and 1940. Measurements were taken, however, only on the fibers that were 1⅛ inches long. The 1⅛-inch length has been used in the writer's uniformity of length work (*13, 14*). Hawkins (*5*) has shown that the long fibers are more immature than those of medium length. The variance of the average measurement of the fibers of each strain was calculated by methods of Fisher (*3*) and Snedecor (*12*). In experiments 1 and 2 the standard deviation of each set of measurements was calculated.

To determine ribbon width, ribbon thickness, and number of convolutions 50 fibers of 4 strains in the 1⅛-inch length group were used. Five measurements of ribbon width and thickness were made on each fiber and these were averaged to obtain the measurement for that fiber. The mean and standard deviations were calculated for each strain and simple correlations were run between each of the characters ribbon width, ribbon thickness, and number of convolutions. The convolutions were counted in a field of 1.6 mm.

TABLE 1.—*Mean swollen-diameter measurements and standard deviations for fibers of different lengths from 6 strains of Acala cotton grown at State College and at Roswell, N. Mex., 1938*

Fiber length (inches)	1064, State College		1064, Roswell		1517, State College		1517, Roswell		1980, State College		Sh 5, State College		2780, State College		2768, State College	
	Diameter	Standard deviation	Diameter	Standard deviation	Diameter	Standard deviation	Diameter	Standard deviation	Diameter	Standard deviation	Diameter	Standard deviation	Diameter	Standard deviation	Diameter	Standard deviation
	Microns	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*
1 3/16	23.6	2.8	23.4	2.5	22.6	1.9	22.7	2.8	20.8	2.5	22.6	4.3	21.6	2.1	22.8	1.9
1 1/4	22.9	2.1	25.1	1.8	23.2	2.2	23.4	3.0	20.9	1.9	22.7	2.4	22.4	2.5	23.0	2.1
1 3/8	23.0	2.2	23.1	2.4	22.6	2.3	22.8	1.9	21.7	2.4	21.9	2.8	22.3	2.2	24.1	2.2
1	23.5	2.0	23.4	2.3	22.8	2.6	23.1	2.4	21.5	2.4	23.0	2.3	22.5	2.1	23.6	2.1
7/8	23.6	2.0	22.6	2.7	23.1	2.6	23.6	2.5	22.3	2.3	22.4	2.9	23.1	2.0	23.6	2.5
3/4	23.9	2.4	24.4	2.4	23.5	3.0	23.6	2.9	23.3	2.5	23.2	2.5	21.7	1.8	23.5	2.1
5/8	24.0	2.5	27.3	3.1	24.9	2.8	24.2	2.5	23.4	2.7	23.1	2.7	23.5	3.1	23.6	2.0
1/2	24.4	2.3	26.2	2.5	26.1	3.6	25.1	3.8	23.4	2.6	27.0	3.3	24.2	2.0	24.0	2.9
3/8	26.4	4.8	25.7	3.0	25.1	3.4	24.1	2.7	23.0	2.3	24.7	4.2	23.4	2.8	23.1	2.8
1/4	24.8	3.7	26.5	2.4	24.7	2.6	23.8	2.7	23.2	2.4	25.5	3.7	23.4	2.2	23.1	2.6
1/8	25.6	4.1	24.5	2.6	24.1	1.8								2.8		

EXPERIMENTAL DATA

SWOLLEN-FIBER DIAMETER

EXPERIMENT 1

The swollen diameters of each fiber length from ⅛-inch to 1⅜ inches of six strains are given in table 1. In figure 1, *A*, is shown a comparison of the mean swollen-diameter measurements of the different lengths of strain 1064 grown at State College and at Roswell, N. Mex. The diameters at State College are more regular and differ in other respects from those recorded for the cotton produced at Roswell.
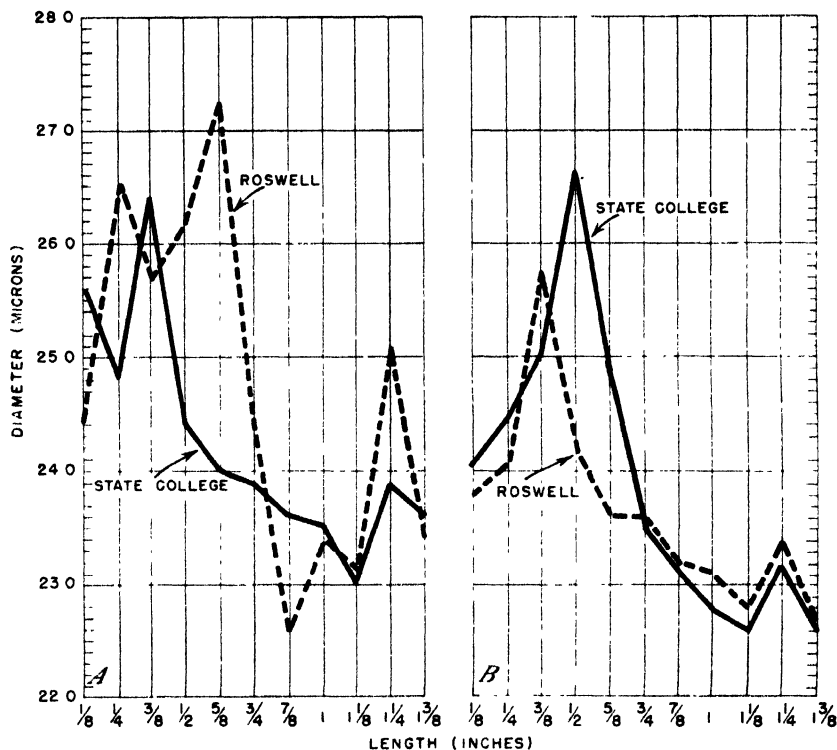
FIGURE 1.— Swollen-diameter measurements of different fiber lengths of strain No. 1064 (*A*) and of strain No. 1517 (*B*) grown at Roswell and at State College, N. Mex.

It will be noted in table 1 that the diameters of the longer lengths are, as a whole, smaller than the diameters of the shorter lengths. The 1⅛-inch length usually has the smallest diameter, and in measuring and comparing strains, the measurement of this length group would make for convenience. In New Mexico, where the cotton-breeding program has as one of its objectives the increase of the proportion of 1⅛-inch fibers in the total fibers of its cotton, interest in this length indicated that it should be used in making diameter measurements.

In strain 1064 the smallest diameter of any length is 23.0µ at the 1⅛-inch length, and the largest diameter is 26.4µ at the ⅜-inch length, a difference of 3.4±0.53, which is highly significant. One

can readily see that with the standard deviation shown, a difference no larger than 1μ would be significant. Except in the case of strain 2768, the diameters tend to become larger with the shortening of the length. The data on the two strains 1064 and 1517, came
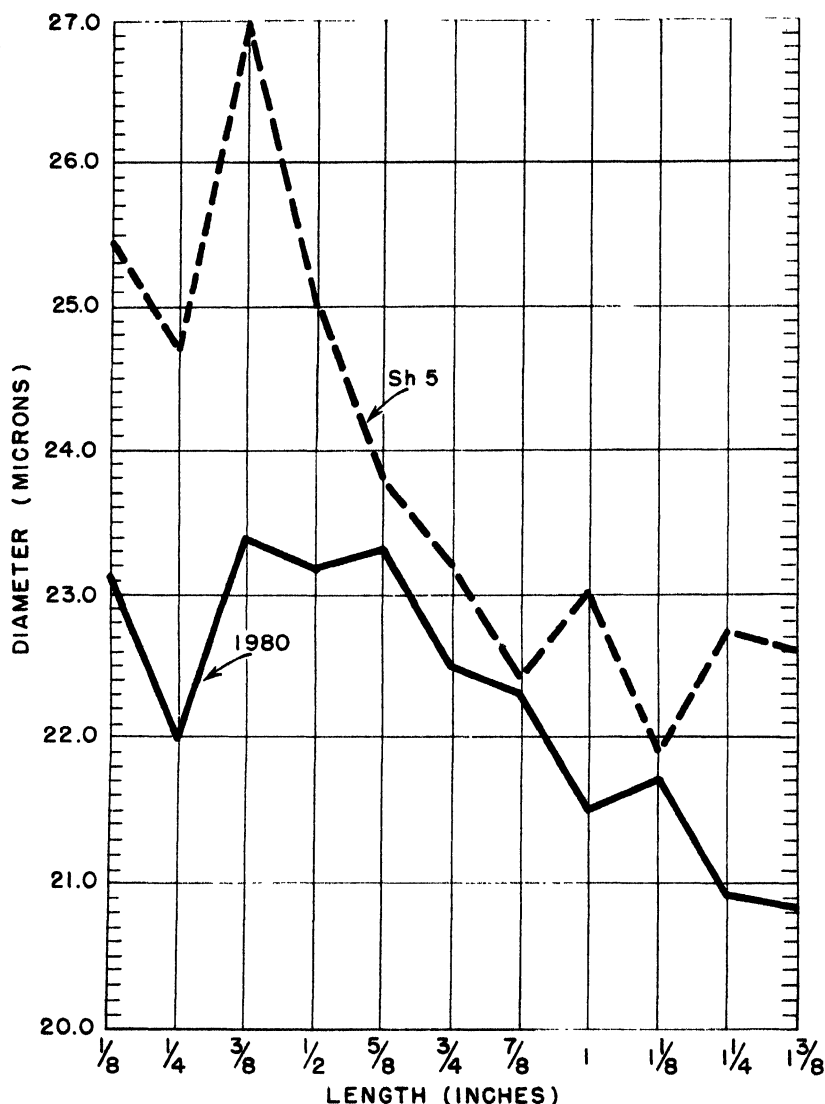
FIGURE 2.—Swollen-diameter measurements of different fiber lengths of unrelated strains Nos. Sh5 and 1980, both grown at State College, N. Mex.

from samples grown at State College and at Roswell. The tests on the other strains in 1938 came from samples grown at State College. Figure 1, *B*, shows a comparison of swollen-diameter measurements of one strain (1517) grown in two localities; the two curves are very similar. Figure 2 shows a comparison of swollen-diameter measurements of two unrelated strains grown in the same locality.

TABLE 2.—*Mean swollen-diameter measurements and standard deviations for 1⅛-inch fibers from each plot of the 12 strains of Acala cotton grown in the 1938 advanced test and of 16 strains of cotton grown in the 1940 advanced test at State College, N. Mex.*

12 STRAINS, 1938 TEST

Strain	Block 1			Block 2			Block 3			Block 4			Average diameter[1]
	Plot No	Mean	Standard deviation	Plot No	Mean	Standard deviation	Plot No	Mean	Standard deviation	Plot No	Mean	Standard deviation	
		Microns	Microns		Microns	Microns		Microns	Microns		Microns	Microns	Microns
1064	11	23.2	2.5	21	22.6	2.2	34	23.0	2.2	48	22.3	3.2	22.8
1080	3	21.8	2.3	24	21.9	2.4	26	21.6	2.4	38	22.0	2.2	21.0
1517	1	22.6	2.3	17	23.9	2.0	36	21.7	1.9	42	22.9	2.3	23.0
8h 5	10	20.6	1.9	18	22.4	2.0	33	20.9	2.4	37	21.1	2.8	22.4
1450	9	22.4	1.8	22	21.9	2.4	25	23.3	1.7	43	22.0	1.9	20.9
1485	8	21.3	2.0	14	23.7	2.6	31	22.6	2.0	44	22.3	2.3	21.9
1493	12	21.9	2.0	16	21.8	2.3	32	22.0	2.2	41	22.1	1.9	22.8
1000	5	22.4	2.8	20	22.5	2.2	30	23.1	2.1	46	22.5	2.4	22.1
1757	7	21.9	2.1	13	22.3	1.8	35	22.0	1.8	45	22.3	1.8	22.4
1497	2	22.4	2.2	19	23.3	2.1	28	22.3	2.4	47	22.5	2.6	22.1
California	4	23.0	2.0	15	23.3	2.4	27	23.1		39	22.3		22.5
N28-5	6	24.3	1.3	23			29			40	22.5		23.3

16 STRAINS, 1940 TEST

Strain	Block 1		Block 2		Block 3		Block 4		Block 5		Block 6		Average diameter[3]
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	
	Microns	Microns	Microns	Microns	Microns	Microns	Microns	Microns	Microns	Microns	Microns	Microns	Microns
2815	21.04	3.19	22.65	2.22	23.95	2.39	21.28	1.92	20.89	2.44	22.78	2.60	22.10
F. S. 1	21.27	2.91	22.62	2.13	23.32	2.08	22.48	2.22	22.17	1.84	22.02	1.76	22.31
2321	21.92	2.73	22.24	2.64	23.43	2.10	21.03	2.48	22.48	2.47	24.00	2.23	22.68
1517	22.51	2.38	23.83	2.40	23.59	2.43	23.82	1.84	23.97	2.22	23.71	2.51	23.57
1493	23.28	2.30	24.60	2.85	24.15	1.95	23.54	2.33	22.57	2.32	23.59	2.44	23.45
F. S. 2	20.65	1.98	23.36	2.31	23.68	2.21	23.13	2.65	23.01	1.87	22.66	1.65	22.75
1450	21.06	2.20	23.70	2.29	22.74	2.35	22.44	1.74	21.88	2.44	20.91	2.28	22.12
F. S. 3	22.51	2.20	23.00	2.33	21.87	2.42	23.92	2.30	23.46	2.24	21.91	2.27	23.18
3231	23.64	1.90	25.00	1.90	23.20	2.12	21.51	2.33	23.86	1.88	22.62	1.60	22.96
2020	22.02	2.40	25.00	1.94	23.95	2.15	24.07	2.05	23.28	2.61	24.30	2.68	22.93
Deltapine 12[4]	24.06	3.24	23.46	2.61	22.99	2.15	23.25	2.15	23.16	1.94	23.73	2.06	24.09
3593	22.96	2.38	23.50	2.11	22.53	1.99	23.87	2.16	21.78	1.83	23.35	1.89	22.64
F. S. 5	22.41	2.05	23.46	2.35	22.72	2.38	24.10	2.09	23.26	1.89	23.73	2.00	23.03
1064	23.32	1.94	23.50	1.98	22.32	1.61	23.46	2.05	21.97	2.84	23.35	1.75	23.79
2314	22.35	1.94	24.04								23.38		23.27
1080	22.92	2.11	23.22		22.66				21.13	1.81	23.07	2.19	22.74

[1] Difference in average diameter required for significance 0.8
[2] Mean of other 3 blocks used.
[3] Difference in average diameter required for significance 0.92
[4] Not an Acala strain; seed derived from E. C. Ewing, Scott, Miss.

EXPERIMENT 2

In table 2 the mean diameter with its respective standard deviation is given for each plot of each strain in the advanced strain test at State College in 1938. In the last column is shown the average diameter of each strain, and in footnote 1, a difference for significance of 0.8μ.[3]

TABLE 3.— *Variance of the mean swollen-diameter measurements of the fibers grown in 1938 and in 1940*

Year and source of variance	Degrees of freedom	Mean squares	F values	Year and source of variance	Degrees of freedom	Mean squares	F values
1938:				1940:			
Blocks	3	0.1015	------	Blocks	5	2.4419	--------
Strains	11	1.6953	5 1	Strains	15	1 6656	2.63
Error	33	.3329		Error	75	.6323	- ----
Total	47	- -----	---------	Total	95	--	----

The variance of the mean swollen-fiber diameters of both the 1938 and 1940 strains are shown in table 3.

The data in table 2 indicate in many cases significant differences between strains. In the 1938 test, strain N28–5 had the largest diameter, 23.3μ, and strain 1450 had the smallest, 20.9μ. This is a difference of 2.4μ, or 11.5 percent of the smallest strain.

Figure 3 presents a histographic comparison of all the measured strains grown at State College in 1938. At the right the strain 1980 mean measurement plus or minus the significant difference is shown for convenience of rating the strains.

The foregoing results suggest that if there are differences of 10 percent or more between related strains, the cotton breeder could improve his strain by using swollen-diameter tests of individual plants before they get through the breeding block. In fact, that is the point in the breeding program at which any test should be made, including the spinning test. It is easy to discard individual plants but costly to throw out a strain after it has been increased for distribution.

The mean diameter with its standard deviation for each plot of the advanced strain test at State College in 1940 is given in table 2. The average diameter for each strain is shown in the last column and the difference required for significance, 0.92, at the bottom of the table. All fibers used in the test, except those from Deltapine 12, were from strains of Acala. Strain 2815 had the smallest average diameter, 22.10μ, while Deltapine 12 had the largest, 24.09μ. The difference between these two, 1.99μ, is significant. The Acala strain having the largest diameter is 1517 with an average diameter of 23.57 or a difference from 2815 of 1.47, which is significant. Both 1517 and 2815 are selections from 1064, but neither 1517 nor 2815 differs significantly in average diameter from 1064. Both 1517 and 1064 have been tested for strength and found to have a strong fiber.

Figure 4 presents a histographic comparison of all the strains grown in the State College advanced strain test in 1940. A greater amount of variation is shown within strains in these data than in those for 1938. The amount required for a significant difference

[3] Standard error of mean difference = 0.408.

between strains was 0.8µ in 1938 and 0.92 in 1940.   The fiber diameters of the 1940 s trains were also larger on the whole than those of 1 938.

RIBBON WIDTH, THICKNESS, AND NUMBER OF CONVOLUTIONS

Data concerning ribbon width, ribbon thickness, number of convolutions, and the correlation relationships of these three characters in four strains are shown in table 4.   Pope (*10, 11*), who studied these
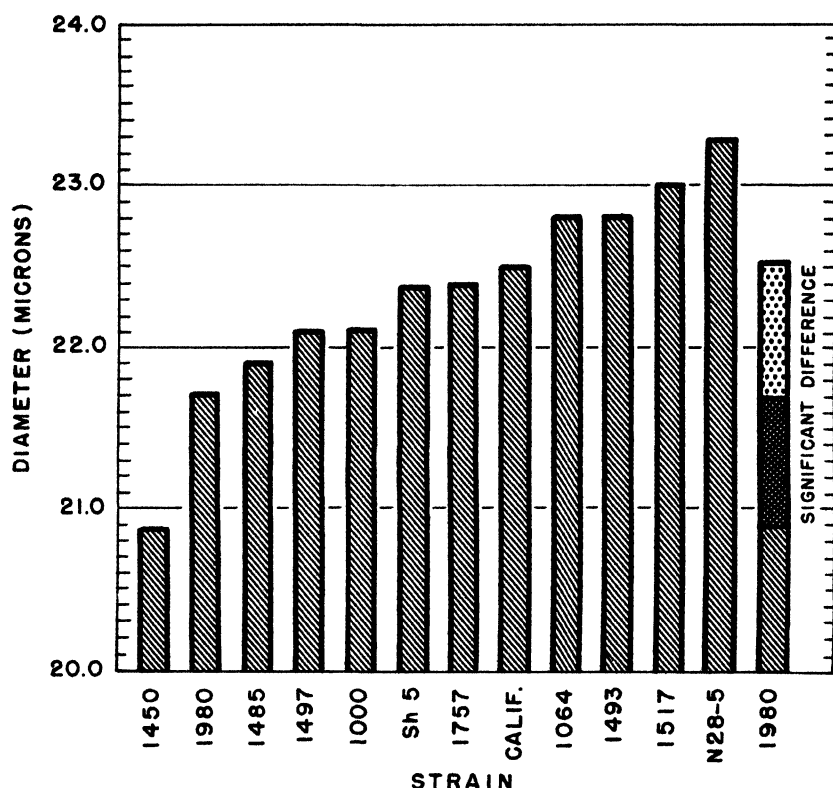
FIGURE 3. -Histogram showing the mean swollen-diameter measurements of fibers of the 12 strains grown in the advanced test in 1938, at State College, N. Mex

characters, recommended a sample size of 100 to 200 fibers, but these data are taken on only 50 fibers.

The difference between the smallest ribbon width, 19.7µ, and the largest, 21.3µ, is 1.6±0.49, which is significant.   Strains 1064 and 1517, seed of which has been released to farmers in New Mexico, differ in width by 0.9±0.47—hardly a significant value.

In thickness there is a significant difference between the smallest and the largest, namely, 0.9±0.31.

In number of convolutions the difference between the highest and the lowest is 1.7±0.73, which can be considered significant.

It is apparent from the foregoing analysis that the relationships as

shown by the simple correlation coefficients are not the same in all four strains. This behavior has also been observed by the writer in
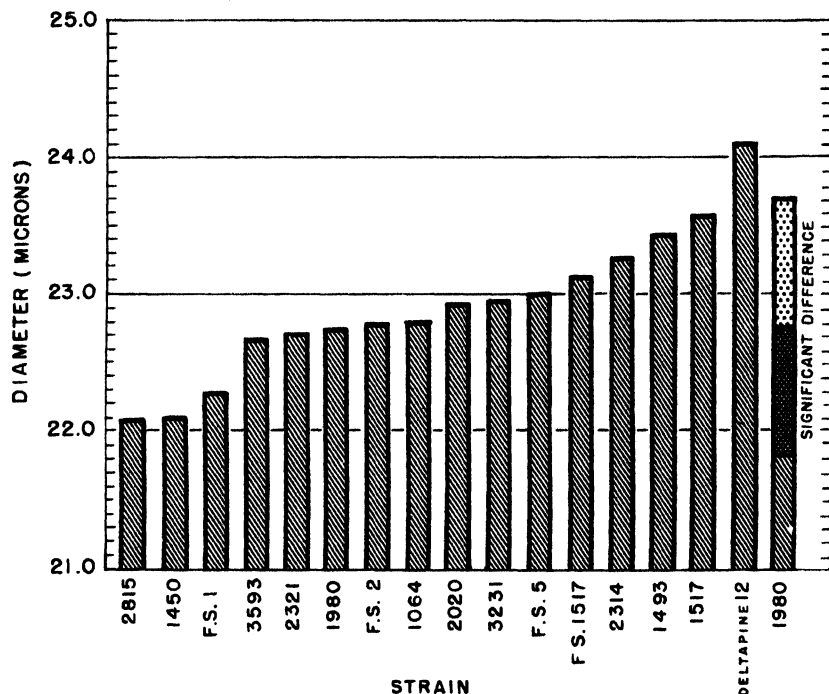
FIGURE 4.—Histogram showing the mean swollen-diameter measurements of fibers of the 16 strains grown in the 1940 test at State College, N. Mex.

a study of the relationship of characters where a large number of characters and strains were considered.

TABLE 4.—*Mean ribbon width, ribbon thickness, and number of convolutions per 1.6 mm.; the standard deviation of each and the simple correlation of ribbon width and thickness, ribbon width and number of convolutions, and between ribbon thickness and number of convolutions of 4 strains of Acala cotton grown in advanced tests at State College, N. Mex.,[1] 1938 and 1940*

Strain No.	Ribbon width		Ribbon thickness		Number of convolutions		Correlation coefficients [2] of—		
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Ribbon width and thickness	Number of convolutions and ribbon width	Ribbon thickness and number of convolutions
	Microns	*Microns*	*Microns*	*Microns*	*Microns*	*Microns*			
1000____	19 7	2.3	11 9	1.3	15.9	3 5	0. 49*	−0. 12	−0.22
1517_____	20 4	2.1	11 1	1 0	16. 7	3.8	.20	−. 58*	−.01
1757_____	20. 8	1.5	11.1	1.2	16. 1	2.1	.20	−. 35*	− 21
1064_____	21. 3	2.6	11 0	1.8	17. 6	3.8	.59*	−. 44*	−. 55*

N=50.
[2] Significant coefficient *.

DISCUSSION

The real object of these studies was to determine whether certain strains could be differentiated on the basis of diameter of fiber, and, if so, whether by the use of simple equipment it would be practicable to incorporate into the breeding program a system of testing progenies for fiber diameter. Swollen-fiber diameter and fiber weight per inch, as measures of fineness (*1, 9*), have been shown to be of importance in spinning value (*6, 7, 16*), but the relation of shape of fiber and fiber weight per inch has not as yet been determined. When the cotton technologist has cotton of uniform fiber weight per inch, uniform diameter, uniform 40° angle, and uniform shape and length of fiber, he will be able to determine accurately the characters of raw fiber necessary to spin certain classes of textiles.

From the results here reported it can be seen that there are significant differences among strains of Acala cotton. It has been shown (experiment 1) that the different lengths of fibers from a given sample have different fiber diameters. For the comparison of strains as to fiber diameter it would not be practical to measure each length group so it seems logical to pick out for measurement one convenient length group. In New Mexico, as has been said, the breeding work on cotton has as one of its objectives the breeding for high uniformity of length of lint. The character used in the testing for this high uniformity of length has been the percentage of 1⅛-inch-plus fibers. In other words, in this program fibers 1⅛ inches long are important. The strains of Acala now distributed to farmers have about 25 percent of their fibers of this or longer lengths. Some new strains and progenies run as high as 60 percent of 1⅛-inch-plus fibers. Therefore at this station the 1⅛-inch group was selected as a suitable and convenient length to use in testing for swollen diameter of fibers. This measure would not, of course, represent the true diameter of the fiber, but it would be a figure that could be used in comparing one strain with another.

As regards ribbon width, ribbon thickness, and convolutions the Indian Central Cotton Committee (*1*) does not now test its standard cottons for these characters, because of the small contribution that these characters make to spinning utility. However, the diameter of fiber should be considered along with its strength, width of cell wall, and fiber weight per inch.

SUMMARY

Data were obtained on six different strains of Acala cotton to determine the swollen diameter of fiber in each group length of 1⅜, 1¼, 1⅛, 1, ⅞, ¾, ⅝, ½, ⅜, ¼, and ⅛ inches. The results showed that the shorter lengths had the largest diameters.

Measurements of swollen diameter on the 1⅛-inch length were made on 12 strains from the advanced test in 1938 and 16 strains in 1940 at the New Mexico station, and significant differences were obtained between strains. In 1938 the strain with the largest diameter was N28–5, with a mean fiber diameter of 23.3μ; strain 1450 had the smallest mean fiber diameter, 20.9μ. The difference between the two strains is 2.4μ, whereas 0.8μ would have been significant. Of all the Acala strains tested in 1940 No. 1517 had the largest mean fiber diameter, 23.57μ. Strain 2815 had the smallest mean fiber diameter,

22.10μ. The difference between these two mean diameters is 1.47, which is significant. This indicates that the cotton breeder could breed strains of cotton with small diameters.

A significant difference was found among strains in respect to ribbon width and thickness, and significant positive correlation coefficients between ribbon width and thickness were obtained in two of the four strains tested; significant negative coefficients were obtained between ribbon width and number of convolutions in three of the four strains, while between ribbon thickness and number of convolutions a significant negative correlation was found in only one of the four strains.

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RELATION OF LENGTH OF PHOTOPERIOD AND INTENSITY OF SUPPLEMENTAL LIGHT TO THE PRODUCTION OF FLOWERS AND BERRIES IN THE GREENHOUSE BY SEVERAL VARIETIES OF POTATOES [1]

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INTRODUCTION

In order to conduct a breeding program for the production of new varieties of potatoes adapted to Nebraska conditions, it became necessary to devise some means of producing flowers and seed in the greenhouse during the winter, for in all parts of the State seed production in the field is undependable even with the most free-blooming varieties, and some varieties never bloom.

By using supplemental electric light until 11:30 p. m., Stevenson and Clark (6)[2] secured extensive blossom and berry production with 20 strains of potatoes (*Solanum tuberosum* L.) in the greenhouse at Arlington, Va., in March and April of 1932. Strains that had set seed naturally in the field in Maine bloomed more readily in the greenhouse than those that had not set seed.

Jones and Borthwick (5) concluded that "the first inflorescence of the potato was differentiated at approximately the same node under a range of conditions including size of seed piece, temperature and photoperiod." They also reported that flower primordia were developed in total darkness.

Clarke and Lombard (3) concluded that 16 hours was a suitable photoperiod at Beltsville, Md., for both flowering and fruiting with a number of potato varieties. However, with a 16-hour photoperiod the mean number of seed balls in the Triumph variety was only 2 per plant and such a free bloomer as Earlaine produced only 6.00 per plant. The supplemental light used had an intensity of only 20 to 30 foot-candles. The weakness of the supplemental light together with the cloudy winter days may account for the failure to obtain more berries on even the continuous-light plants, which with Earlaine averaged only 5.89 seed balls per plant. In view of their results and those of Jones and Borthwick (5), Clarke and Lombard concluded that the failure of flowers to develop in short photoperiods is due to the abscission of young buds.

Edmundson (4) reports that in the relatively bright winter days at Greeley, Colo., plants that emerged on January 10 or 12 in two winters bloomed and set seed in a constantly increasing quantity as

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the photoperiod was increased from 9 to 11, 13, and 17 hours. However, the maximum average number of seed balls for 10 varieties was only 6.15 per plant and the maximum percentage of pollinated flowers that produced seed balls was only 56. This low set might have been due to the 17-hour photoperiod being too short or the supplemental light (20 to 30 footcandles) being too weak, or both. Photoperiod did not alter the percentage of fertile pollen.

REVIEW OF SEED PRODUCTION IN NEBRASKA

The first attempt to produce potato seed in the Nebraska Experiment Station greenhouse at Lincoln was made in the winter and spring of 1937. The potatoes were planted the middle of January. The average light intersity outside the greenhouse during daylight hours for the entire period prior to the opening of the first blossoms in late March and early April, was about 300 gm.-cal. per square centimeter per day. This was supplemented with artificial light (about 25 footcandle intensity at the tops of the plants) to make a total photoperiod of 16 hours. Berries were produced by 32 lines of potatoes, flowers of which were fertilized with pollen from several good pollen-producing lines. Plants growing in gravel with a balanced nutrient solution produced more flowers and berries than those growing in a rich soil in ground beds. Many failures occurred when flowers were pollinated during the very hot days which occurred frequently after mid-April. Consequently few berries were obtained from the numerous flowers produced in late April and May.

In order to avoid the disastrous consequences of these very hot days in April and May, plantings were made on January 1 in 1938 and first blossoms were produced in early March. The mean daylight intensity for each month in gram-calories per square centimeter per day was as follows: January 190, February 243, March 333, and April 485. The mean light intensity for 40 days prior to the opening of blossoms was about 250 gm.-cal. (January 20–March 5). Supplemental light of about 25 foot-candles was used to make a 16-hour photoperiod. Under these conditions berries maturing during May were produced on 67 lines. This was still not early enough for the breeding program. Seed was needed that would germinate promptly when planted in mid-August and thus permit the seedling tubers to be harvested in February and complete their rest period in time for field planting in June.

Some of the writer's observations and the findings of Borthwick and Parker (*2*) suggested that long photoperiods might be used for only a relatively brief period prior to the induction of flower buds and also that more light during the photoperiod might improve flower production. During the winter of 1938–39, 12 varieties or clones of potatoes were planted on December 9, and the plants emerged during the period of shortest days, December 21 to 25 (*9*). Half of these plants were given supplementary light of 100 to 125 foot-candles at the plant tops for an 18-hour photoperiod and the other half only 30 to 40 foot-candles. The mean intensity of the natural daylight from emergence of plants to pollination of first flowers was 226.2 gm.-cal. per square centimeter per day. With the brighter supplemental light 3 varieties set berries quite readily, a fourth set very few, but 8 set none at all. With the weaker supplemental light only 2 varieties produced berries satisfactorily, 1 produced a very few, and the other

9 produced none.   Another set of these lines was started on December 24, emerging about January 10.   The daylight intensity from emergence to pollination of first flowers was higher than with the other set, namely, 254 gm.-cal. per day.   The supplemental light for the 18-hour photoperiod was only of 30 to 40 foot-candle intensity.   With this slightly brighter daylight but weak supplemental light, 4 varieties produced berries in a fairly satisfactory manner and 6 of the 12 varieties produced some berries.   The results with these 3 sets of plants indicated that a long photoperiod was not in itself sufficient for flower and berry production, but that more light during the photoperiod either from brighter sunshine or brighter supplemental light was necessary.

In 1938–39, when supplemental light of 30 to 40 foot-candle intensity was used, extensive blooming occurred with a 24-hour photoperiod, but a 16-hour photoperiod was not satisfactory and a 13-hour photoperiod was very inadequate for even the most free-blooming varieties.   With these same plants a high level of nitrogen nutrition was necessary for best flower and pollen production.

With early strain Triumph plants a few flowers were produced when a 16-hour photoperiod (20 to 30 foot-candles of supplemental light) was used for 35 days following plant emergence on February 1, 1939.   When 16-hour days were used for less than this time, none of the flowers attained maturity and most of them dropped off.   Either these days were too short or light was too weak to bring about floral induction.

In the winter of 1939–40 early and midseason strains of Triumph potatoes planted on December 29 were supplied with 18- or 24-hour photoperiods during intervals increasing by 10 days from 20 to 70 days after the sprouts had been started in the light in a humid chamber (8).   In a parallel series sprouting occurred in the dark (in sand), and supplemental light which started 10 days after plant emergence was continued for 10 to 40 days.   The midseason strain produced berries more readily than the early one, and both produced more with 24- than with 18-hour photoperiods.   Best berry production occurred when long photoperiods were used continuously until the first few flowers were pollinated.   Long days during the last few weeks prior to blooming appeared to be essential for satisfactory berry production.   Long photoperiods during the sprouting period and first few days thereafter appeared to exert some beneficial effect measurable as increased numbers of berries.   The supplemental light used was between 100- and 200-foot-candle intensity at the tops of the plants.

These earlier endeavors indicated quite clearly that either greater light intensity or longer days must be used in order to obtain berry production during the winter with most varieties or lines of potatoes.   The work during the winter of 1939–40 was planned to determine the effect of greatly increased light intensity during 18- and 24-hour photoperiods (throughout all or part of the life of the plant) upon the production of berries both by varieties that produce berries easily and by those that seldom fruit.

EXPERIMENTAL METHODS

The four varieties selected and some of the specific flowering characteristics considered in choosing them were the following:

Earlaine.—Weakly vegetative early variety that produces numerous fertile flowers when most other varieties drop their flowers or produce only primordia.

Triumph 12.—The earliest clonal strain of Triumph potatoes that has been selected in Nebraska. It does not produce flowers unless conditions are very favorable, and generally it produces little or no pollen, although at several earlier times pollen suitable for crossing and selfing has been obtained from it. No pollen was produced by this strain in any treatment of this experiment. Abscission of partly developed flowers was most common with this clone. It does not bloom as freely as the later maturing clonal lines of Triumph potatoes.

B4–1.—A hybrid secured from Dr. F. A. Krantz and listed by him as Minnesota 29.32–1–34. It blooms quite readily and produces more pollen per anther than almost any other variety. The percentage of viable pollen was found to be somewhat lower than that of Katahdin, but this is of little consequence in view of the volume of pollen produced.[3]

Katahdin.—This variety produces an abundance of flowers with excellent pollen under a wide range of conditions.

Seed pieces of early harvested tubers were planted in sand on November 1, 1939. On November 21, 1939, when most sprouts were 2 to 5 cm. long, the sprouting seed pieces were carefully removed, sorted as to size, and held in shallow basins of water until replanted. Two sprouting seed pieces were planted in fine gravel in a 10-inch pot. One seed piece placed to the south was covered only 1 cm. in order to facilitate the later removal of stolons and tubers so as to retain carbohydrates in the tops. However, blooming was so satisfactory in the better treatments that tuber removal was not resorted to. The north seed piece in each pot was covered to a depth of 10 cm. The tops of most of these north sprouts were up to or above the surface by November 25, which is considered as the emergence date. Six pots of two seed pieces each were planted with each of the four varieties in each of eight light treatments. The pots of one treatment were arranged in three east to west rows with eight pots per row. All pots were placed tightly against each other. A 20-inch space was provided between treatments. Because of the crowding of the plants within one treatment and the consequent unequal illumination of all but the upper leaves, the plants of each variety were placed in a different position in each row. The positions of the plants of each variety were as follows:

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North row                          B T E K B T E K
South row___                      _ E K B T E K B T
Center row___                       T E K B T E K B
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(E, Earlaine; K, Katahdin; B, B4–1, and T, Triumph 12.)

The gravel in the pots was flooded one to three times a day, according to the amount of transpiration, by raising a 5-gallon reservoir of nutrient solution connected to the pots with tubes in the bottom of each pot. After about 30 minutes the gravel was flooded and the reservoirs were lowered to a level sufficient to maintain about 2 inches of solution in the bottom of each pot. This level was maintained by adding sufficient water daily to fill all pots completely when the reservoirs were raised. Nutrient solution was made up from commercial salts according to the formula listed as 2D by Withrow and Biebel, (*10*), 45 gm. of the dry mixed salts being used to each 5 gallons of water. The solution was drained and renewed once each week. Solution reservoirs were covered with boards to reduce evaporation and prevent contamination by algae (fig. 1).

Three degrees of supplemental light intensity were secured by using 300-, 150-, and 60-watt bulbs. These bulbs were arranged over each

treatment on a framework that supported two rows of five bulbs each. The bulbs were suspended 2 feet above the pots when the experiment was started. They were raised at intervals as the plants grew so that they remained at this distance above the tops of the plants with a reasonable degree of constancy. In order to utilize electricity most efficiently, each bulb was provided with a circular reflector the inside

FIGURE 1. Plants used for treatment 8, showing method of growing plants in gravel in 10-inch pots with nutrient solution supplied from 5-gallon cans, through tubes entering the bottoms of pots; method of arranging lights so that they can be raised as plants grow; position of thermograph; black curtains for separating light treatments (one curtain drawn); and white sheets of paper used for several weeks to reflect light on to plants. Panels along side bench were made of sisal kraft paper painted with aluminum paint and were raised at night (as shown) to protect seedling plants of another project from the long photoperiod. The 5-gallon cans were raised on to the trestle to flood pots with nutrient solution and were then lowered so that nutrient solution was maintained at a depth of about 2 inches in the bottom of the pots. One can supplied eight pots Cans were covered with a board to prevent growth of algae in the solutions.

of which was painted with aluminum paint (this reflected approximately 10 percent more light than white lead paint). In the earlier weeks white sheets of paper were suspended from the sides and ends of the light-supporting frames so as to reflect light back on to the plants and also to prevent absorption by the black dividing curtains. These devices increased the light from 40 to 60 percent. As the plants grew, the use of these sheets of paper became complicated and only the circular reflectors were continued. Mean values for this supplemental light, as reported in table 1, were secured from readings made with a Weston meter at the tops of the 48 plants of each treatment. Readings were made each time just before and just after the lights were raised. In the course of the experiment 145 sets of light readings or almost 7,000 individual readings were secured. The occasional very high mean light readings recorded were due to the fact that the tips of a few plants grew very close to the bulbs. With some plants

located between the lights the readings were low before the lights were raised because the tops grew above the zone of bright light. Very few flowers developed after February 1 when the last readings were made.

In order to secure photoperiods of the definitely desired lengths, lights were turned on at 7 a. m. and left on till the daylight was brighter than that coming from the electric lights, which was generally between 8:30 and 9:30 a. m. On several very dark days the lights were left on throughout the day. On most days only the brightest light treatment gained by such midday supplemental light, and the weakest light treatment gained only during the dim-light portions in the morning and evening. In the late afternoon, lights were generally turned on between 4:30 and 5 p. m. For the 18-hour photoperiod lights were automatically turned off at 1 a. m. Black curtains were drawn between treatments involving either different light intensities or different photoperiods. These curtains were drawn across when lights were turned on in the late afternoon, but in the morning when the lights were left on to supplement weak daylight, the curtains were drawn back.

TABLE 1.—*Photoperiod, mean light intensity, and mean temperature used with various treatments*

Treatment No.	Photoperiod	Power of each of 10 light bulbs used per treatment	Mean supplemental light intensity, Nov. 23 to Feb. 1	Initiation or period of supplemental light	Mean temperature at tops of plants, Nov. 24 to Mar. 30
	Hours	*Watts*	*Foot-candles*		°*F.*
1	24	300	481	Nov. 21	63 9
2	24	300	493	Nov. 1	
	24	300	501	Nov. 21–Dec. 18	
3	18	60	72	Dec. 19–Jan. 6	
	24	300	509	Jan. 7–Jan. 15	
	9 5–12 4		0	Jan. 16	
4	24	150	216	Nov. 21	63. 6
5	18	150	200	do	
6	18	300	515	do	62. 9
7	24	60	65	do	
8	24	60	58	Nov. 1	57. 5

Because of the great amount of heat generated by the large light bulbs, it was impossible to maintain uniform temperatures for all treatments. In order to secure similar temperatures, fans were suspended just below the bulbs at one end of each of the light frames supporting 300-watt bulbs. By blowing the air from the cooler side of the greenhouse across the tops of the plants, the average temperature there was lowered 6° to 10°. As a result of the extensive ventilation which was necessary at night to maintain a generally low temperature in the greenhouse, the temperature in the vicinity of the weaker lights averaged about 6° F. lower than that under the brighter lights. As the day temperatures were almost the same in all parts of the greenhouse, these differences in the temperature for the entire period resulted from the higher temperatures that occurred during the supplemental lighting period. Daytime maximum temperature never exceeded 70° on cloudy days and exceeded 75° only during a few days of bright sunshine. Thermographs were suspended from the light frames of four treatments so that they recorded the temperature near the tops of the plants and always at the same distance below the lights. The mean daily and weekly temperatures were secured from thermograph sheets by means of a planimeter. The weekly means are shown in figure 2, and the seasonal means in table 1.

With daily supplemental lighting, the most extensive flower development occurred in late December and early January when the natural day length was only between 9.2 and 9.6 hours and the daily light intensity, registered a short distance from the greenhouse (*1*), averaged between 145 and 210 gm.-cal. per square centimeter during the various weeks (fig. 3).   Under these conditions other potato plants of a num-
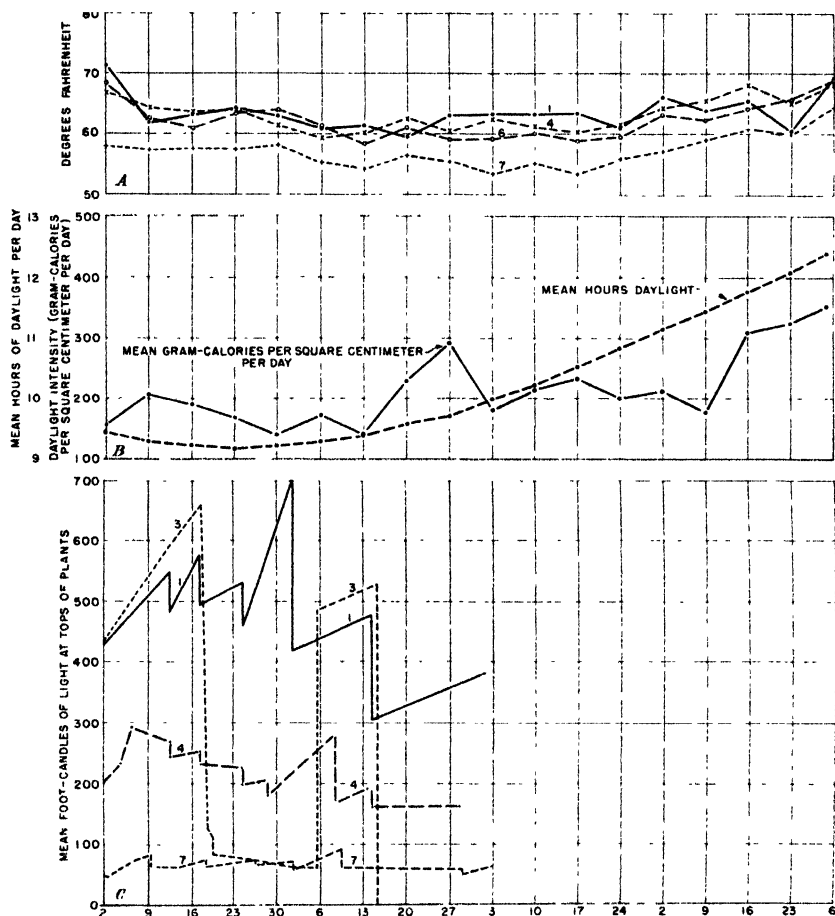
FIGURE 2.—Mean temperature, by weeks, in the vicinity of the tops of plants of each of four treatments described in table 1 (*A*), mean hours of daylight and weekly mean gram-calories per square centimeter per day represented by the daylight intensity (*B*), and the intensity of the supplemental light (foot-candles) at the tops of plants of each of four treatments listed in table 1 (*C*).

ber of varieties growing in the same greenhouse without supplemental light were typical of short-day plants described in an earlier paper (*7*); i. e., they had a single axis without axillary shoots and the inflorescence never developed beyond the primordial stage.

Every flower was pollinated at or just before anthesis with pollen from flowers of the three pollen-producing varieties; the pollen used was from flowers in the same light treatment.   All relevant informa-

tion was noted on the tags attached to each flower at the time of pollination. Notes concerning abortion of flowers or degree of development of primordia were recorded for each cluster when the developing berries were bagged. Berries were harvested when ripe, during March and April. All seed was weighed by clusters after having been extracted by fermentation, washing, and drying.

Some estimate of the vegetative growth was secured by measuring the height of each plant a few days after it emerged and at weekly intervals until February 7, when the growth of the third axis was complete with practically all plants.

Just prior to the termination of the project an effort was made to determine relative foliage development. Four terminal leaflets from each plant were removed, and the leaf outlines were traced on sheets
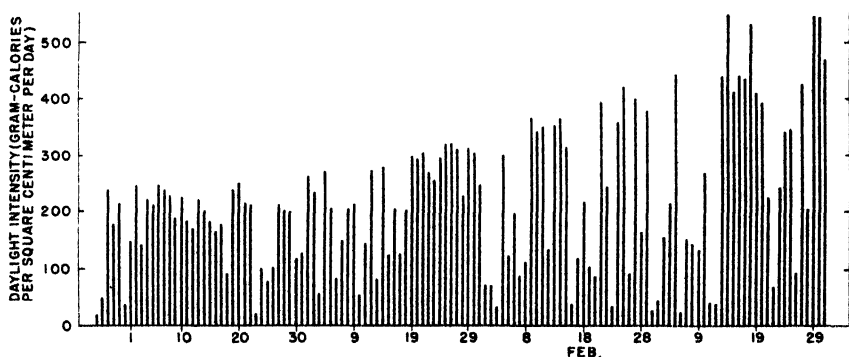
FIGURE 3.—Intensity of natural daylight at Lincoln, Nebr., in gram-calories per square centimeter per day for each day of the experiment, 1939–40.

of paper placed on a translucent glass over a light. Later the leaf areas were calculated by means of a planimeter. After the berries were harvested the plant tops were cut off at the surface. The length and number of nodes in each axis and in the underground stem parts were recorded for each plant. The number of tubers weighing more than 5 gm. and the total number and weight of tubers were recorded by plants.

EXPERIMENTAL RESULTS

VEGETATIVE GROWTH

A brief consideration of the nature of the vegetative growth is essential for understanding the differences in blooming habits of the varieties under the different treatments. Plants grown either with the lowest supplemental light intensity or the longest photoperiod (24 hours) made the most rapid growth of any and grew to be the tallest plants (fig. 4). The reduction in growth rate when berries were developing was most noticeable with the plants grown in a 24-hour photoperiod with the brighter light—the treatment that produced the heaviest crop of berries (treatment 1). In contrast, plants grown with the weakest supplemental light continued to grow at a rapid rate practically all season (treatment 7).

The length of each axis increased as the supplemental light intensity decreased (table 2, fig. 5). A reduction in length of photoperiod
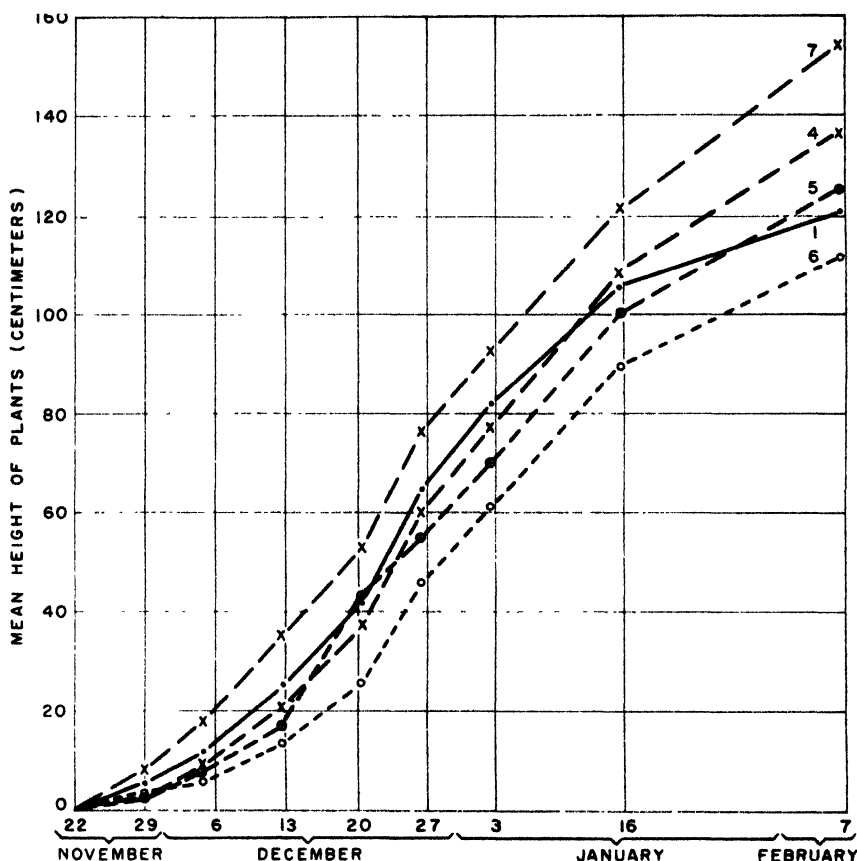
FIGURE 4.—Mean height of plants of all varieties in each of five treatments listed in table 1 at various dates till vegetative growth was terminated at the third axis by pruning.

brought about little difference in the central axes, but caused lateral axes to be much shorter. The lateral axes were less than half as long as the central axes. The 18-hour photoperiod with brightest light (treatment 6) produced the shortest plants. These plants were darker and more uniform than those of any other treatment (fig 5). Linear stem growth was greatest in Katahdin, followed in order by B4–1, Triumph 12, and Earlaine.

The mean size of the apical leaflets was found to increase with each decrease in light intensity or decrease in length of photoperiod (table 3). The leaflet area of various varieties arranged in increasing order was as follows: B4–1, Katahdin, Triumph 12, and Earlaine.

TIME OF BLOOMING

Within the range of this experiment, time of flower production was advanced more by increasing the intensity of the supplemental light than by extending the photoperiod (fig. 6). The development of sprouts in light with a 24-hour photoperiod hastened blooming in Earlaine, B4–1, and Katahdin, but delayed it in Triumph 12 when
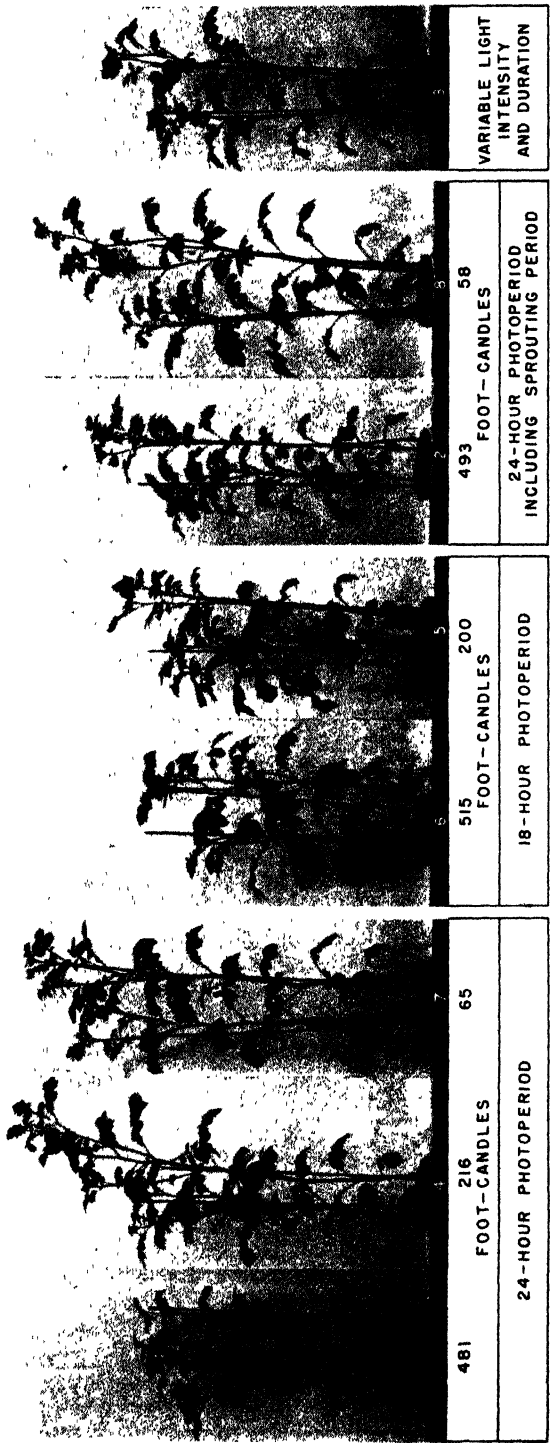
FIGURE 5.—One Triumph 12 plant from the north row under each light treatment listed in table 1 as it appeared on January 20.

the brightest light, was used (treatment 2 vs. treatment 1). With the weakest light blooming was delayed in all varieties (treatment 8 vs. treatment 7). Under the latter condition the status of Triumph 12 was uncertain because of the small number of blossoms with both treatments.

TABLE 2.—*Mean length of axes of the plants of all varieties in each treatment*

Treatment No.	Photo-period	Mean supple-mental light intensity, Nov. 23 to Feb. 1	Length of—			Total length
			Central axis	First lateral	Second lateral	
	Hours	*Foot-candles*	*Centimeters*	*Centimeters*	*Centimeters*	*Centimeters*
1	24	481	75	37	41	153
4	24	216	86	41	47	174
7	24	65	100	42	48	190
6	18	515	75	28	29	132
5	18	200	86	33	33	152
2 [1]	24	493	80	31	36	147
8 [1]	24	58	87	45	45	177
3 [2]	(2)	(2)	80	34	40	154
All treatments			84	36	40	160

[1] Sprouts developed in 24-hour photoperiod of given light intensity.
[2] Photoperiod and light intensity altered during life of plant, see table 1.

TABLE 3.— *Mean area per leaflet of systematically chosen apical leaflets from plants of all varieties in each treatment*

Treatment No.	Photo-period	Mean sup-plemental light inten sity, Nov 23 to Feb 1	Mean area per leaflet for—				Mean, all varieties
			Earlaine	Triumph 12	B4-1	Katahdin	
	Hours	*Foot-candles*	*sq. cm.*	*sq. cm*	*sq cm.*	*sq. cm.*	*sq. cm.*
1	24	481	27 3	22 1	19 4	20 6	22 3
4	24	216	29 9	32 3	23 8	26 6	28 1
7	24	65	37 5	32 5	29 2	29 1	32. 1
6	18	515	31 6	30. 7	26 6	31 7	30 2
5	18	200	35 1	33 7	28 3	33 5	32. 8
2 [1]	24	493	31 4	22.3	20. 6	20 8	23 7
8 [1]	24	58	28 0	31 1	27. 2	29 5	29 0
3	(2)	(2)	27 6	28 3	24 3	23 8	26 0
Mean			30 9	29 3	24 9	27. 0	28 0

[1] Sprouts developed in 24-hour photoperiod of given light intensity.
[2] Photoperiod and light intensity altered during life of plant, see table 1.

The mean dates of pollination for the flowers of all varieties produced in a 24-hour photoperiod in the order of decreasing light intensity were January 14, 21, and 25. The mean pollination dates for bright and intermediate light with an 18-hour photoperiod were January 20 and 23 respectively.

NUMBER OF PRIMORDIA AND FLOWERS

Fewer primordia and flowers were developed in the second than in the first inflorescence, and still fewer were developed in the third. The number of undeveloped primordia was about the same in all inflorescences, but the number of flowers diminished rapidly with each succeeding inflorescence.

The number of discernible floral primordia differentiated by three inflorescences per plant increased noticeably with a 24-hour photo-
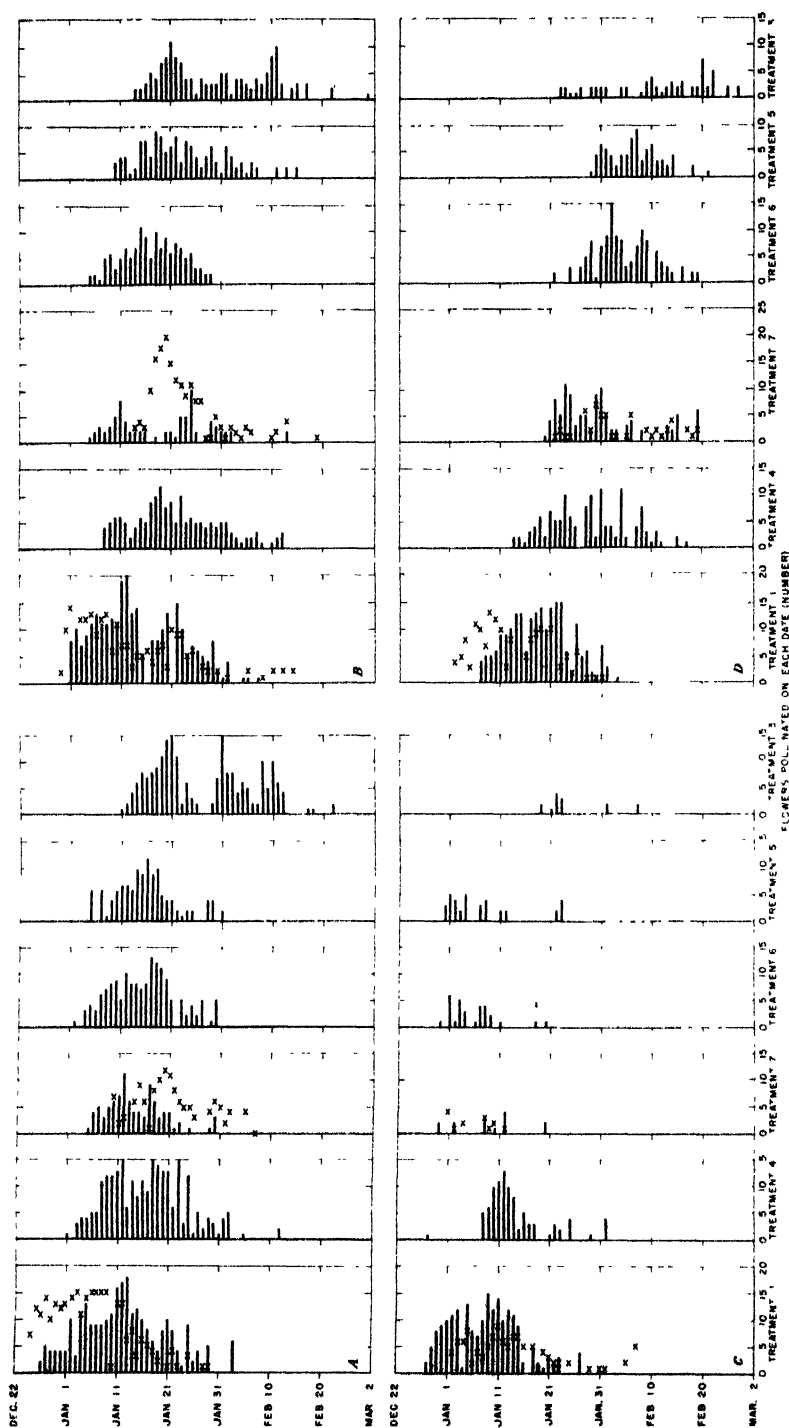
FIGURE 6.—Number of flowers of each variety, pollinated on each date, undergoing treatments listed in table 1. The "x" marks in treatments 1 and 7 indicate the extent of flowering on each date with treatments 2 and 8 respectively. These latter treatments involved development of sprouts in continuous light of high and low intensity: *A*, Earlaine; *B*, Katahdin; *C*, Triumph 12; *D*, B4–1.

period, but was not altered appreciably by differences in light intensity (table 4). The number of primordia in the first inflorescence of each plant was generally smaller with the lowest than with the highest supplemental light used, having averaged 11.7 per cluster for the former and 13.5 for the latter. Fewer primordia failed to develop to flowers as the light intensity was increased (table 4). Primordia abortion generally decreased slightly as the photoperiod increased.

The number of flowers sufficiently developed for pollination was greatest when the brightest supplemental light was used for the 24-hour photoperiod (table 4). The effectiveness of the 24-hour photoperiod in producing good flowers was greatly enhanced by using the brightest light. A 24-hour period with weak supplemental light was relatively ineffective for floral production. In earlier work light intensities of 30 to 40 foot-candles used to make 16- to 18-hour photoperiods were found to be still more inadequate in producing flowers.

With the scant-blooming Triumph 12, the number of flowers produced by using the brightest light in a 24-hour photoperiod was so much greater that all other treatments appeared relatively ineffective. With free bloomers such as the other three varieties, supplemental light of half the intensity was relatively effective with a 24-hour photoperiod. However, with the 18-hour photoperiod, reasonably satisfactory flower production occurred only with Earlaine and Katahdin. These two varieties also bloomed quite well in the variable light treatment (treatment 3).

TABLE 4.—*Mean number of floral primordia differentiated, primordia dropping, flowers pollinated, berries harvested, and mean weight of berries and of seed per plant of each variety in each treatment*

TOTAL NUMBER OF FLORAL PRIMORDIA DIFFERENTIATED

Treatment No.	Photo-period	Mean supplemental light intensity, Nov. 23 to Feb. 1	Total on 3 clusters, mean of 1 plant for—				All varieties
			Earlaine	Triumph 12	B4-1	Katahdin	
	Hours	*Foot candles*	*Number*	*Number*	*Number*	*Number*	*Number*
1	24	481	29	29	24	31	28.2
4	24	216	38	26	24	30	29.5
7	24	65	29	24	27	25	26.2
6	18	515	26	21	21	27	23.7
5	18	200	24	24	21	28	24.3
2¹	24	493	29	26	27	30	28.0
8¹	24	58	25	23	25	32	26.2
3	(²)	(²)	29	27	21	32	27.3
All treatments			28.6	25.0	23.8	29.4	26.7

TOTAL NUMBER OF PRIMORDIA DROPPING

1	24	481	9	13	8	9	9.7
4	24	216	19	20	13	17	17.3
7	24	65	22	23	18	19	20.5
6	18	515	14	19	13	15	15.2
5	18	200	14	22	15	18	17.3
2¹	24	493	10	17	13	11	12.8
8¹	24	58	16	21	16	18	17.7
3	(²)	(²)	14	26	17	22	19.8
All treatments			14.8	20.1	14.1	16.1	16.3

TABLE 4.—*Mean number of floral primordia differentiated, primordia dropping, flowers pollinated, berries harvested, and mean weight of berries and of seed per plant of each variety in each treatment*—Continued.

TOTAL NUMBER OF FLOWERS POLLINATED

Treatment No.	Photo-period	Mean supplemental light intensity, Nov. 23 to Feb. 1	Total on 3 clusters, mean of 1 plant for—				All varieties
			Earlaine	Triumph 12	B4-1	Katahdin	
1	24	481	20	16	16	22	18 5
4	24	216	19	6	11	13	12 2
7	24	65	7	1	9	6	5.7
6	18	515	12	2	8	12	8.5
5	18	200	10	2	6	10	7.0
2¹	24	493	19	9	14	19	15.2
8¹	24	58	9	2	9	14	8.5
3	(²)	(²)	15	1	4	10	7.5
All treatments			13.8	4 9	9.6	13 4	10.4

TOTAL NUMBER OF BERRIES PRODUCED

Treatment No.	Photo-period	Light intensity	Earlaine	Triumph 12	B4-1	Katahdin	All varieties
1	24	481	15.8	13 7	12.3	17.8	14.9
4	24	216	17 5	4 8	9 5	11.5	10.8
7	24	65	6 4	5	5.1	7 4	4.8
6	18	515	10 4	1 2	7.5	11 4	7 6
5	18	200	8.3	1.1	4 8	9.1	5 8
2¹	24	493	15.8	1.7	11 6	16.9	11 5
8¹	24	58	7 7	1 0	8 3	10 3	6.8
3	(²)	(²)	12 0	7	2.5	6 9	5 5
All treatments			11.7	3.1	7 7	11.4	8 5

WEIGHT OF BERRIES

Treatment No.	Photo-period	Light intensity	Earlaine *Grams*	Triumph 12 *Grams*	B4-1 *Grams*	Katahdin *Grams*	All varieties *Grams*
1	24	481	111 6	109 5	78 2	147 6	112 3
4	24	216	122 5	42 3	66 0	84 8	78 9
7	24	65	42 1	5 3	34 0	65 5	36 7
6	18	515	74 8	9 8	53 0	87.7	56 3
5	18	200	53 3	8 8	41 8	60 3	41.0
2¹	24	493	114 3	49 9	66 0	123.8	88.5
8¹	24	58	46 5	7.8	58 8	84 3	49 3
3	(²)	(²)	81.2	5 2	17.3	59.2	40 7
All treatments			81.0	29 9	51 9	89 2	63.0

AIR-DRY WEIGHT OF SEED

Treatment No.	Photo-period	Light intensity	Earlaine	Triumph 12	B4-1	Katahdin	All varieties
1	24	481	1 94	1.67	.98	1.84	1 60
4	24	216	2 16	.62	.75	1.06	1.15
7	24	65	64	.09	.48	71	48
6	18	515	1.19	.12	.60	.98	.72
5	18	200	.78	14	49	.76	.55
2¹	24	493	1 83	75	79	1.59	1 24
8¹	24	58	83	13	75	1.05	.69
3	(²)	(²)	1 39	08	.19	.66	.58
All treatments			1.34	.45	.63	1.08	.88

¹ Sprouts developed in 24-hour photoperiod of given light intensity.
² Photoperiod and light intensity altered during life of plant. see table 1.

In all varieties some pollinated flowers failed to develop into berries. These failures are attributed to the fact that although pollinated before the corolla opened and once or twice thereafter (with all flowers present), some of the flowers abscissed. In some instances, especially in very large inflorescences, the development of the berries from the latest flowers was inhibited by the rapid growth of berries that were initiated earlier. The loss of flowers at this stage was much smaller than prior to the period of anthesis. The percentage of pollinated

flowers that dropped off without producing berries increased with each succeedingly later inflorescence. The number dropping was much the same with all varieties (difference between fourth and third sections of table 4).

The actual number of flowers dropping varied by treatments and on a percenatge basis it was much greater with the shorter photoperiod or lower light intensity.

NUMBER OF BERRIES

The number of berries per plant decreased greatly as the supplemental light intensity decreased or the photoperiod was shortened (table 4). With Earlaine and Katahdin reasonably satisfactory berry production took place with the two brightest light intensities and with both photoperiods. However, in the case of Triumph, the only acceptable treatment for berry production was the 24-hour photoperiod with brightest supplemental light, and with this treatment the berry production of Triumph compared very favorably with that of Earlaine and Katahdin (fig. 7). The B4-1 line was almost as dependent on abundant illumination as the Triumph. Earlaine exhibited the most versatility for blossom and berry production as it produced a good crop of berries with the variable light treatment (treatment 3) with which the berry crops of Triumph and B4-1 were practically failures. Apparently bright light is needed until flowers are well developed if a satisfactory crop of berries is desired. Starting sprouts in continuous light (treatments 2 and 8) did not materially influence the number of berries. About three-fourths of the berries were produced in the first clusters. With the least fruitful treatments no berries were produced in third clusters of some varieties. In fact, in such cases the third inflorescences were very poorly developed.

WEIGHT OF BERRIES

The total weight of berries was altered by treatments in much the same manner as total number of berries, having been increased by increases in both photoperiod and light intensity (table 4). The size of individual berries varied greatly and no differences in mean weight per berry could be established for treatments. However, differences in size of berries produced by different varieties were relatively great. The mean weights of berries in all treatments of each variety (calculated from the fourth and fifth sections of table 4) were as follows:

	Weight in grams
Triumph	9. 66
Katahdin	7. 82
Earlaine	6. 92
B4-1	6. 74

AIR-DRY WEIGHT OF SEED

Again, with each decrease in light intensity or duration of photoperiod there was a pronounced decrease in the weight of seed produced by each treatment (table 4). With the light of greatest intensity during a 24-hour photoperiod, plants of the four varieties produced two to more than three times as much seed as those with the weakest light. Increasing the length of the photoperiod from 18 to 24 hours more than doubled the weight of seed. The longer day increased the weight of seed relatively more than it did the weight of the berries.

The brightest light treatment with a 24-hour day exerted the most pronounced influence with the Triumph, the seed weight of which was increased about twentyfold above that of the weakest light. With

FIGURE 7.—Two Triumph 12 plants which underwent treatment 1 described in table 1, showing type of vegetative growth and several clusters of berries.

the variable light (treatment 3), seed production was fair with Earlaine, poor with Katahdin, and very poor with Triumph and B4-1.

There was more difference between varieties in the weight of seed per weight of berry than between treatments, Earlaine having the

greatest seed weight per berry weight and Triumph next; between the other two varieties there was little difference (calculations made from the fifth and sixth sections of table 4).

TUBER PRODUCTION

With the low temperatures of this experiment good tuberization occurred with all treatments. The mean number of tubers produced in all treatments was 8.7 per plant and the mean total weight was 241 gm. Differences between treatments were too small and irregular to warrant comment. The most significant difference was between varieties. Triumph consistently produced the greatest number and weight of tubers and Earlaine the smallest. The average production per plant for all treatments was 327 gm. with Triumph and 143 gm. with Earlaine.

GENERAL CONCLUSIONS

As a result of this work and previously published work which is cited, it appears that a long photoperiod is essential to induce satisfactory seed production during the winter with the Triumph variety and others that do not bloom readily. Seed production is improved by using relatively intense supplemental light. This increased intensity of supplemental light is more important when the natural daylight is inferior in quality and of short duration. Bright light and a long photoperiod must be used throughout the life of the plant, or at least during the greater part of it. Stated in another way—it seems that a minimum daily amount of light is needed throughout the life of the plant for flower and seed production and if much of this occurs as natural daylight, less need be applied supplementally. Furthermore, while a long photoperiod appears highly desirable, it can be compensated to a considerable extent by an increase in light intensity. A very long photoperiod with very bright supplemental light can be depended upon to produce flowers and seed.

SUMMARY

The experiment herein reported was planned to determine the relative effectiveness for the production of potato seed in the greenhouse, of different intensities of supplemental light with 18- and 24-hour photoperiods, of intermittent use of long photoperiods, and of the sprouting of parent seed tubers in continuous light.

The most extensive blossom, berry, and seed production took place when a light intensity of about 500 foot-candles at the tops of the plants was used for supplemental light during a 24-hour photoperiod. This treatment was effective with varieties that do not bloom or set fruit readily. The amount of blooming and berry production with all varieties decreased as the photoperiod or light intensity decreased.

The effectiveness of a 24-hour photoperiod for increasing the number of berries was greatly enhanced by a little more than doubling the supplemental light intensity, i. e., increasing it from about 200 to about 500-foot candles. When the intensity of natural daylight was low, the use of supplemental light of only 60 foot-candles intensity in a 24-hour photoperiod was practically of no value for producing seed with varieties that do not bloom very readily.

When long photoperiods of bright supplemental light were used early in the life of the plant and supplemental light was used only at

intervals thereafter, blooming took place better with varieties that bloom easily than when weak supplemental light was used every day, but this method was practically a failure with varieties that do not bloom readily.

The number of primordia, blossoms, and berries was greatest on the first clusters, least on the third or last. The degree of development of the second and third clusters was dependent upon increase in day length and light intensity.

The production of sprouts in continuous light exerted some influence on time of flowering, but did not affect the production of berries.

The vegetative growth was most rapid and extensive with the plants that received the weakest supplemental light for a 24-hour period. Slowest and least vegetative growth occurred with an 18-hour photoperiod with the brightest supplemental light. The length of each axis was increased by decreasing the supplemental light. The length of the central axis was not altered by the photoperiod, but the length of the lateral axes increased as the photoperiod increased.

The average size of leaves increased as light intensity or photoperiod decreased.

Good tuber development took place with all treatments. In tuber production differences between treatments were not significant but variety differences were relatively great.

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ORIGIN OF THE SUBERIZED SEMIPERMEABLE MEMBRANE IN THE CARYOPSIS OF MAIZE [1]

By Helen Johann [2]

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INTRODUCTION

The suberized semipermeable membrane in the caryopsis of the Gramineae has for the most part been considered to be derived from the integuments rather than from the nucellus. There has been little agreement or detailed evidence offered, however, in regard to the origin of the membrane, which lies immediately within the pericarp (pl. 1, *A–C*) in the mature kernel of corn (*Zea mays* L.). In some of the past histological work, even the presence of this membrane has not been mentioned. This lack of uniformity, in all probability, results from differences in technique employed by the various workers. Suberin and cutin have so little affinity for the histological stains ordinarily used that in the early stages in the development of the caryopsis the membrane, which is then very thin, is not easily identified by color differences. In sections of the mature kernel stained with Delafield's haematoxylin or Flemming's triple stain, the suberized membrane is seen as a hyaline line between the pericarp and aleurone layer (pl. 1, *C*), but at that stage in the development of the kernel it is much too late to determine the origin of the membrane. Earlier, there is a period in the development of the caryopsis when the membrane might appear to be a nucellar structure. This occurs after the disintegration of the integument and before the collapse of the cells of the epidermis of the nucellus. At that time the membrane adheres to and follows the contour of the outer surface of the nucellus (pl. 1, *D*). This stage also is too late to be useful in determining the origin of the membrane. Only when both the inner integument and the epidermis of the nucellus are present and separate is it possible to discover the original location of the primary membrane with any degree of certainty.

In some of the more recent work of this nature, staining difficulties have been avoided by the use of microchemical methods. By means of Sudan III or Sudan IV thin layers of suberin or cutin that otherwise might be overlooked can be demonstrated in the young caryopsis (pl. 2, *A, B*). Further indication of the nature and occurrence of the membrane may be obtained by treatment of the sections with sulfuric acid, in which suberin and cutin are insoluble (pl. 2, *C*).

The object of the investigation reported herein was to determine whether suberization of integumental origin occurs in the corn kernel.

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(275)

REVIEW OF LITERATURE

A review of recent papers dealing with the histology of the caryopses of various Gramineae in which microchemical staining methods were used indicates that suberization of the surface of one or both of the integuments is of common occurrence as compared with suberization of the nucellus.

Andersen (1),[3] using microchemical methods "suggested by Eckerson, Tunmann, and Molisch" in her study of *Poa pratensis* and *P. compressa*, after following the development of the caryopsis, stated (1, p. 1014) that in the mature caryopsis—

the cell walls of the inner integument adjacent to the nucellus form a comparatively thin layer of suberin. The two rows of cells of the inner integument have collapsed somewhat, forming a narrow layer against the suberized layer of the outer integument. A layer of suberin is all that remains of the outer integument. The suberized layers of the inner and outer integuments are so tightly compressed that their identity can be seen only where the seed coat is torn in sectioning.

Eckerson, working with Harrington and Crocker (5), found suberin in the walls of the inner integument in both Johnson grass and Sudan grass, and the authors stated (5, p. 219) that—

the coverings of the naked mature caryopses of both grasses consist of the fused pericarp and the inner integument, the outer integument and nucellus having entirely disappeared, with the possible exception of a portion of the latter over the micropyle.

In sections of the wheat kernel stained with Delafield's haematoxylin or Flemming's triple stain, Pugh, Johann, and Dickson (9) found a thin colorless membrane on the inner side of the inner integument and one of considerable and variable thickness on the outer side. The latter was thin over the embryo. They stated (9, p. 625):

Both membranes stain with Sudan III and are relatively resistant to sulfuric acid, indicating the presence of suberinlike or cutinlike substances.

Using Sudan III or Sudan IV on sections of barley kernels, Tharp (13) found that at flowering time, when as yet there was no structural contact between the integument and nucellus, there was a very faint trace of cutin on the inner and outer epidermis of the outer integument and a distinct cutin membrane on both surfaces of the inner integument, but none at that stage on the epidermis of the nucellus. By the time the embryo had reached the four-cell stage, the nucellar epidermis was adherent to the inner integument and the cutin membrane on the inner surface of the inner integument seemingly was common to both integument and nucellus. In the mature barley kernel the inner integument had a thin inner cutin membrane and a much thicker outer cutin layer of variable thickness, being very thin over the embryo, where it constituted an area of decreased resistance to permeation.

In the maize kernel also, Tharp found that the portion of the seed coat covering the embryo was more easily permeable to normal trichloracetic acid than the part covering the endosperm and that there were differences in the degree of permeation among varieties of corn.

Orton (8), in an earlier study of the permeability of seed coats of sweet and dent corn to mercury compounds, found that the rate of permeability to each of the four mercury compounds used also varied with the corn varieties.

[3] Italic numbers in parentheses refer to Literature Cited, p. 282.
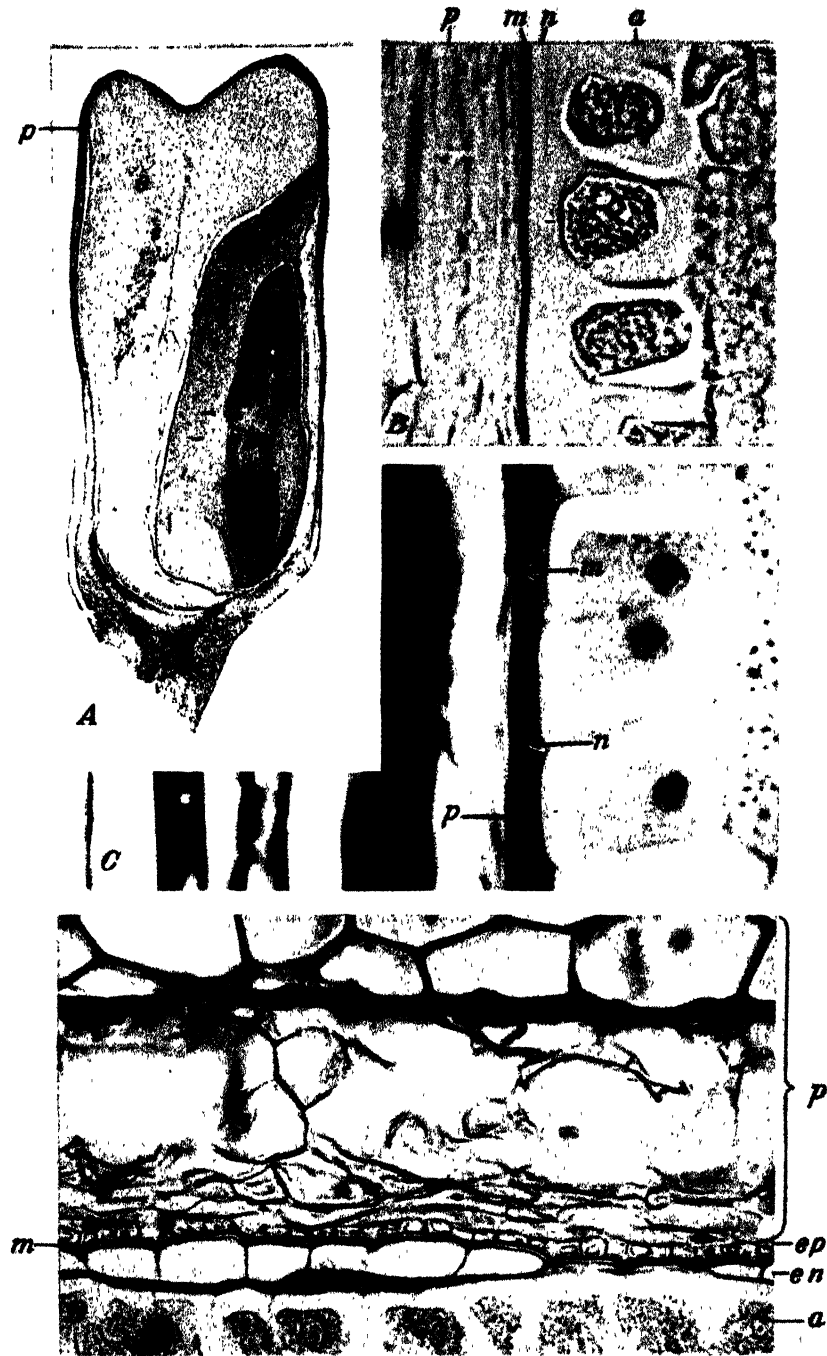
A, Photomicrograph of a longitudinal section of a kernel of inbred line L harvested
September 15, 1931, 42 days after pollination. Paraffin section stained with
Flemming's triple stain. × 8.

B, Portion of a section near the crown of the kernel shown in *A*, showing posi-
tion and thickness of the suberized membrane. Section stained with Sudan
IV, mounted in Karo. × 600.

C, Portion of section shown in *A*, midway of the kernel. In this section, stained
with Flemming's triple stain, the suberized membrane appears as a hyaline line.
× 600.

D, Portion of a cross section of an immature kernel of strain A1237, open-pol-
linated, fixed September 6, 1930. Stained with triple stain. The integuments
have practically disappeared and the hyaline membrane follows the contour
of the remaining cells of the epidermis of the nucellus. × 440.

p, Pericarp; *e*, endosperm; *em*, embryo; *m*, suberized membrane; *a*, aleurone cells;
ep, inner epidermis of the pericarp; *en*, epidermis of the nucellus; *n*, crushed
remains of nucellus.

Identification of suberized membranes. Portions from approximately the same position in three sections of a developing caryopsis of inbred A48 fixed August 29, 1930, 15 days after pollination. $\times$ 480.

A, Stained with triple stain. The membrane is not easily identified by color differences at this stage of development.

B, Stained with Sudan III, mounted in glycerin. Membranes on both surfaces of the inner integument are easily identified. It should be noted that photomicrographs of sections of young caryopses sometimes give the impression of greater thickness in the red-stained membrane than is actually the case, because of a more or less three-dimensional view, due to the constant change of direction of the membrane and the depth of the section.

C, Stained with Sudan III and treated with sulfuric acid. All cellular structure has disappeared; only the suberized membranes retain their identity.

p, Pericarp; *ep*, inner epidermis of pericarp; *ii*, inner integument; *m*, suberized membrane; *en* epidermis of nucellus; *e*, endosperm.
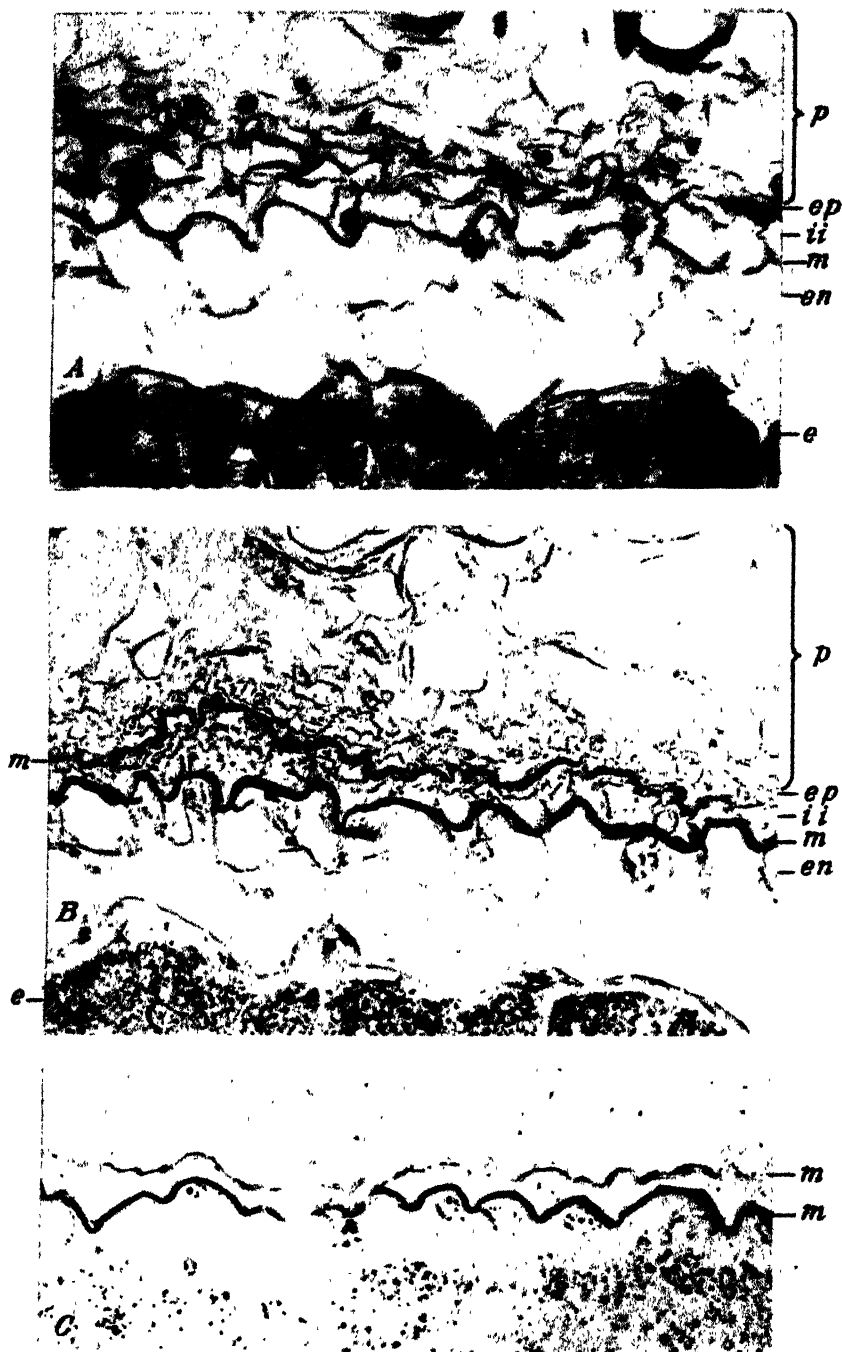
Beeskow [4] stated that the testa of the corn kernel is the layer that is directly responsible for the exclusion of nonpermeating substances. He found fatty and lipoid materials present and concluded that "the semipermeable character of the seed coat is tied up with a lipoid layer and a peculiar granular condition."

Johann (6), working with paraffin sections of young caryopses of corn, pictured a thin suberized membrane stained with Sudan III along the inner surface of the inner integument in contact with the nucellus, and observed (6, p. 866) that—

attempts to demonstrate the presence of suberin on either of the adjacent walls when the epidermis of the nucellus and the inner integument were separable were unsuccessful. Thus, the origin of the membrane may be open to question. It seems probable, however, that the suberized layer in the corn kernel is derived from the integument, as is the case in wheat * * *, blue grass * * *, and Johnson grass * * *, although the number and position of the layers vary with the different genera.

Randolph (11, p. 912) outlined the course of development of the caryopsis of corn as follows:

The major morphological changes in the transformation of the pistil into the mature caryopsis involve (1) the complete displacement of the ovule and integument tissues by the embryo and endosperm, (2) the formation of a suberized membrane derived from the epidermis of the nucellus, and (3) the transformation of the ovary wall into the pericarp.

He also stated (11, p. 913):

A suberized nucellar membrane derived from the outer wall of the nucellar epidermis was present in the mature caryopsis as a continuous, well-defined layer between the aleurone and the pericarp.

In describing the methods used, Randolph stated that paraffin sections were stained either with Flemming's triple stain or Dela-field's haematoxylin, and added (11, p. 883) that—

freehand sections of fresh and fixed material also were utilized, and in making determinations of the composition of the cell wall Sudan IV and certain other stains were employed.

However, the photomicrographs presented show only sections stained with Delafield's haematoxylin.[5]

The methods by which the earlier workers arrived at their conclusions were not always stated in detail. None of the following authors cited has indicated that stains specific for suberin or cutin were used.

True (14), in 1893, studied the development of the caryopsis in corn, wheat, and oats. He noted the strongly cutinized outer walls of the nucellar epidermis of the corn kernel, adding (14, p. 217), "This cuticle is conspicuous in all stages of growth by reason of its great affinity for staining fluids." On the other hand, in speaking of the outer layer of the inner integument of the wheat kernel, he observed (14, p. 222) that "the hyaline outer layer is not easily demonstrated * * *."

Guérin (3), in 1899, described the development of the caryopsis in a number of the Gramineae. He may have seen the membrane in

⁴ BEESKOW, H. C. THE SELECTIVE SEMIPERMEABILITY OF THE SEED COAT OF CORN. 1924. [Unpublished master's thesis, Univ. Chicago]
⁵ Information received in a letter from Dr Randolph

the corn kernel, though his report is not clear on this point and sheds little light on the origin of the membrane. He stated (*3, p. 7*):

Du nucelle il ne persiste bientôt plus que l'épiderme, et le tégument externe de l'ovule disparaît totalement. Un peu plus tard, le tégument interne subit le même sort.

* * * * * * *

Quant au tégument séminal, il n'est représenté que par une bande comprimée dans laquelle il est impossible de retrouver la moindre structure cellulaire.
L'épiderme du nucelle a de même complètement disparu.

Weatherwax (*15, p. 150*) considered that "the protective covering of the grain of corn consists of the *testa* and the *pericarp*. The former is the remnant of the integuments of the ovule * * *." In a later paper (*16*) he states that the nucellus is destroyed and none of it remains at maturity.

Randolph (*10*) studied sections of corn kernels stained with Delafield's haematoxylin and failed to note the membrane. She stated (*10, p. 6*):

The absorption of the tissue in question was followed in a close series of stages, and in all the mature kernels studied there was in most cases no nucellar or integument tissue, the aleurone layer of the endosperm lying in close contact with the inner epidermis of the pericarp. In some scattered places a little unabsorbed material was seen, but it never formed a definite layer.

Haddad (*4*), also using Delafield's haematoxylin, listed the resorption of the tissues in the sweet corn kernel in the following order: Outer integument, inner integument, epidermis of nucellus, and the inner half of the ovary wall. He, also, did not recognize the presence of a suberized membrane.

MATERIAL AND METHODS

The yellow dent corn upon which this work is based was grown at Madison, Wis., in the summers of 1930, 1931, 1938, 1939, and 1940. The inbred lines used in the early years were the Illinois lines L, A48, A1237, and BR10; in 1938 and 1939, A48, Wis. 6, Wis. 26, Wis. R3, Ind. B2, and Ohio 56; and in 1940, a 5-year inbred from Funk's 176A, and Lan, a 7-year inbred from Lancaster Surecrop. Some ears were hand-pollinated, some were open-pollinated. Collections were made at intervals from 2 to several days during the early development of the caryopsis. Of the killing solutions used, chromacetic and a modification of Nawaschin's fluid were generally satisfactory. The specimens were embedded in paraffin by the alcohol-chloroform method.

Sections of the embedded material were stained with Flemming's triple stain or Delafield's haematoxylin to show the cellular structure of the caryopsis, and with Sudan III or Sudan IV to identify suberized or cutinized layers. Some of the latter sections were also treated with sulfuric acid as a further means of demonstrating the presence of suberin or cutin (pl. 2, *A–C*).

No attempt was made to determine the chemical nature of the membrane beyond the tests mentioned above, to which both suberin and cutin give a positive response. Eames and MacDaniels (*2*) stated that a thin cuticle is present sometimes even on the inner epidermis of the ovary and on the integuments of the ovules of vascular plants and added (*2, p. 36*), "In origin the cuticle is probably

Suberization of the inner integument and nucellus.

A, Freehand section of a young caryopsis of 176A, open-pollinated, harvested August 6, 1940. All silks were still green. The inner integument and nucellus, separated for a short distance, show membranes on both surfaces, the one on the inner integument being the more highly suberized. Stained with Sudan IV, mounted in glycerin. × 160.

B, Membrane shown on the inner surface of the inner integument on the germinal side of a young caryopsis in a freehand section of inbred Wis. 6, open-pollinated, harvested August 1939. A slight change in focus would have shown the presence of a thin membrane on the surface of the nucellus also. × 150.

C, Suberin layer on the surface of the inner integument and of the nucellus where they have been separated. Paraffin section of young caryopsis of inbred A48 fixed August 17, 1938. Stained with Sudan III, mounted in glycerin. × 725. In all the sections (*A–C*) the cells of the inner integument appear to be normal and functioning.

p, Pericarp; *ep*, inner epidermis of pericarp; *oi*, outer integument; *ii*, inner integument; *m*, suberized membrane; *en*, epidermis of nucellus; *n*, nucellus.
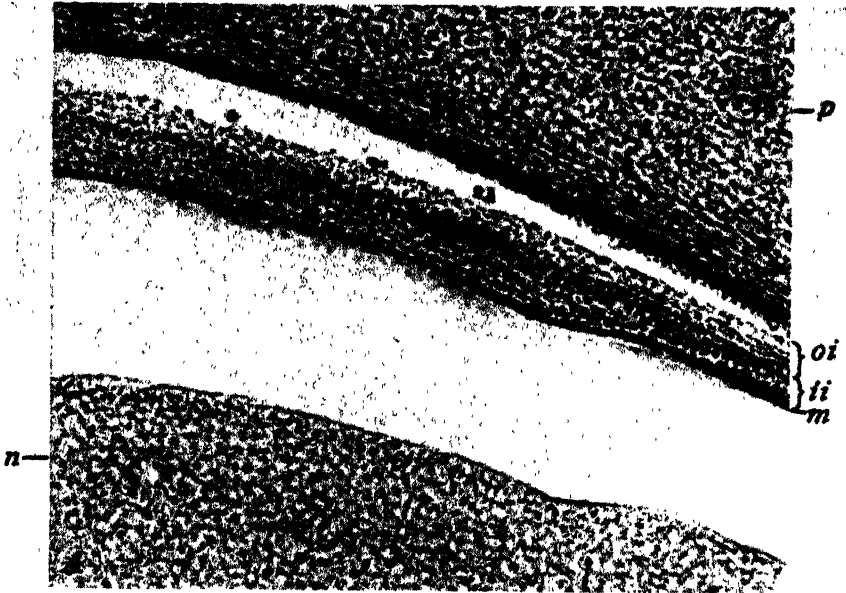
FOR EXPLANATORY LEGEND SEE NEXT PAGE.

a secretion of the protoplast of the epidermal cells * * *." Tharp (*13*) used the terms "cuticle" and "cutin" in relation to the membranes in barley kernels.   On the other hand, Andersen (*1*) and Eckerson (see *5*) each noted suberized layers in the caryopses of the grasses that they studied.

In 1939 and 1940 fresh material was sectioned, stained with Sudan III or Sudan IV, and mounted in glycerin or Karo (*7*).   These free-hand sections, cut without the aid of a microtome, supplemented the embedded material and, despite their obvious shortcomings, presented several advantages.   They could be examined on the day of collection; separation of the nucellus and inner integument was shown more often; and the integuments were usually in a better condition than in the paraffin sections.   Early stages in the formation of suberin were also more easily demonstrated, for apparently the fatty material deposited along the cell wall and not yet consolidated into suberin [6] had been removed from the paraffin sections to some extent by the chloroform used in the embedding process.

With the exception of plate 1, *A–C*, the figures presented are photomicrographs of cross sections of caryopses in early stages of development.   Cross sections were preferred to longitudinal sections because in the former the cells of the inner epidermis of the pericarp presented a greater contrast in size and shape to those of the integuments and thus more sharply defined the outer limits of the integuments.

THE SUBERIZED SEMIPERMEABLE MEMBRANE

Sections of young caryopses of maize, fixed a week or more after pollination and stained with Sudan III or Sudan IV, usually show what appears to be a single thin red membrane between the inner integument and the epidermis of the nucellus, uniting the two surfaces and seeming to be common to both.   From such sections it is difficult to determine the origin of the membrane.   A little earlier, however, it is possible to find sections showing suberization in cases where the nucellus and inner integument are separated for longer or shorter distances.   These sections indicate that suberization of both surfaces occurs (pl. 3, *A-C*), the primary membrane being formed as a thin layer on the inner surface of the inner integument well before degeneration of the adjacent cells of the inner integument has taken place and before suberin is found on the surface of the nucellus (pl. 4, *A, B*).

Degeneration of the inner integument begins on the germinal side of the young caryopsis.   In cross sections of a developing kernel fixed 6 days after pollination, complete collapse of the inner integument for approximately 200μ on the germinal face in the region of the developing endosperm is shown (pl. 5).   For the same distance the suberized membrane is very thin.   Beyond that region the inner row of cells of the inner integument appears to be intact and functioning (pl. 5, *A* and *B*).   As shown in plate 5, *C*, the membrane is thicker and more highly suberized in the region where the adjacent cells of the integument still persist.   Such sharply marked differences

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[6] Rhodes (*12*, *pp. 462-463*), after his study of the chemical nature of the suberin membrane in potato, concluded that "the suberin lamella of the cork cell arises by changes taking place in the fatty material resulting in the appearance of bodies no longer soluble in fat solvents.

"A relatively constant proportion of the soluble fatty substances never undergoes this change and the soluble component of the suberin lamella is mainly responsible for its staining properties."

as these in the integument are not evident in the epidermis of the nucellus, nor does this difference in thickness of the membrane occur in sections nearer the apex of the caryopsis, where as yet there has been no collapse of the inner row of cells of the inner integument.

Further evidence of the integumental origin of the primary membrane is seen in sections of young caryopses in which folding of the inner integument has occurred. Here the membrane follows the inner surface of the integument and in places is far removed from any contact with the nucellus (pl. 6, *A-D*). Folding of the inner integument was most pronounced in inbred 176A but was seen also in Lan and A48.

Later and more variable is the appearance of suberin on the outer surface of the inner integument in the abgerminal region and along the sides of the developing kernel. No instance of a complete coverage of the outer surface has been observed. The patches are thin, and vary in size and location (pls. 2, *B*, *C*; 7, *A*). They may be present in some strains of corn and absent in others. No suberization of the outer integument was observed in the specimens examined.

DISCUSSION

Changes in the developing caryopsis are rapid in the days immediately following fertilization. Randolph (*11*) has outlined in some detail the sequence of events in the degeneration of the inner integument in his material. His description applies in general to any of the inbreds used in the present study, but it does not take into account the suberization of either the inner or outer surface of the inner integument at any stage of development. He considers the suberized semipermeable membrane to be entirely a nucellar structure, and states (*11, p. 908*), "Disintegration of both integuments is well advanced before the nucellar epidermis becomes extensively suberized." The material examined in the present study fails to support this view.

Although degeneration usually begins in the inner integument earlier than in the epidermis of the nucellus, reference to plates 3 to 7 shows that no valid argument can be raised against the integumental origin of the suberized membrane if it is based on the inability of the inner integument to produce such a layer because of the prior degeneration and resorption of its cells.

A membrane is shown to be present on the inner integument earlier than on the nucellus (pl. 4). For this reason and because of its greater thickness during the time the integument and nucellus may be separated it is considered to be the primary membrane. After the two tissues have become physically united it is difficult to determine the subsequent contribution of fatty material made by each toward increased thickness of the membrane, which remains thin at best.

Variations in the thickness and permeability of the membrane in different areas in the mature kernel correspond with changes that have taken place in the integument. In the mature kernel the membrane is most permeable in the embryo region, the region where degeneration of the integument began (pl. 5). It is less so on the sides and in the abgerminal area, where the integuments persisted for a longer time and where the primary membrane is probably reinforced,
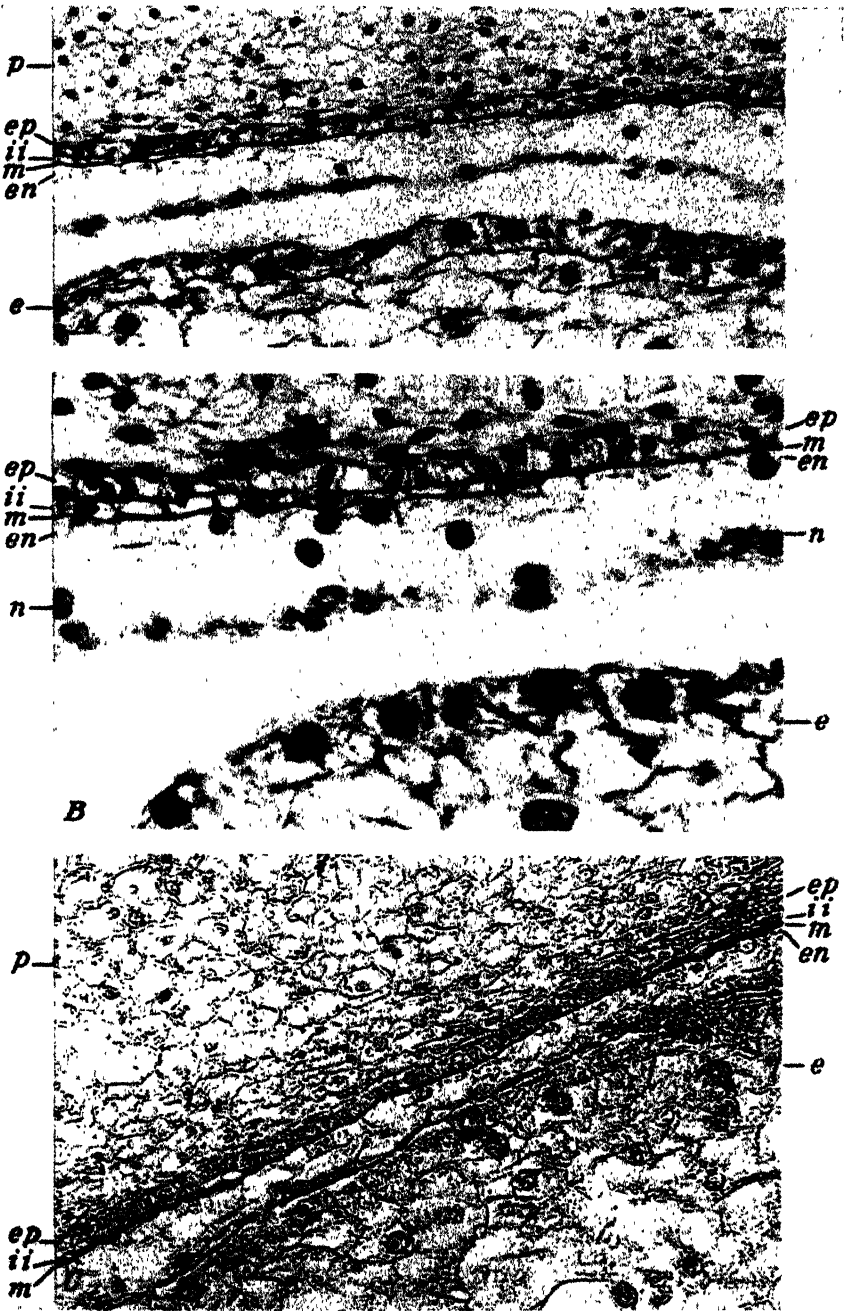
Portions of paraffin sections of inbred A48 fixed August 23, 1938, 6 days after pollination, showing the membrane to be thin in the germinal region where the inner integument has collapsed.

A, In the center of the section complete collapse of the inner integument has occurred; on each side one row of cells of the integument remains. Stained with triple stain. × 240.

B, Detail of the section next in the series to the one in A, showing nuclei in remaining cells of the inner integument. × 460.

Section from the next slide of the series shown in A and B, stained with Sudan III, mounted in glycerin. The membrane is more highly suberized in the region where the inner row of cells of the inner integument still persist than in the area where complete disintegration of the integument has occurred. × 240.

Pericarp; *ep*, inner epidermis of pericarp; *ii*, inner integument; *m*, suberized membrane; *en*, epidermis of nucellus; *n*, remains of nucellus; *e*, developing endosperm.
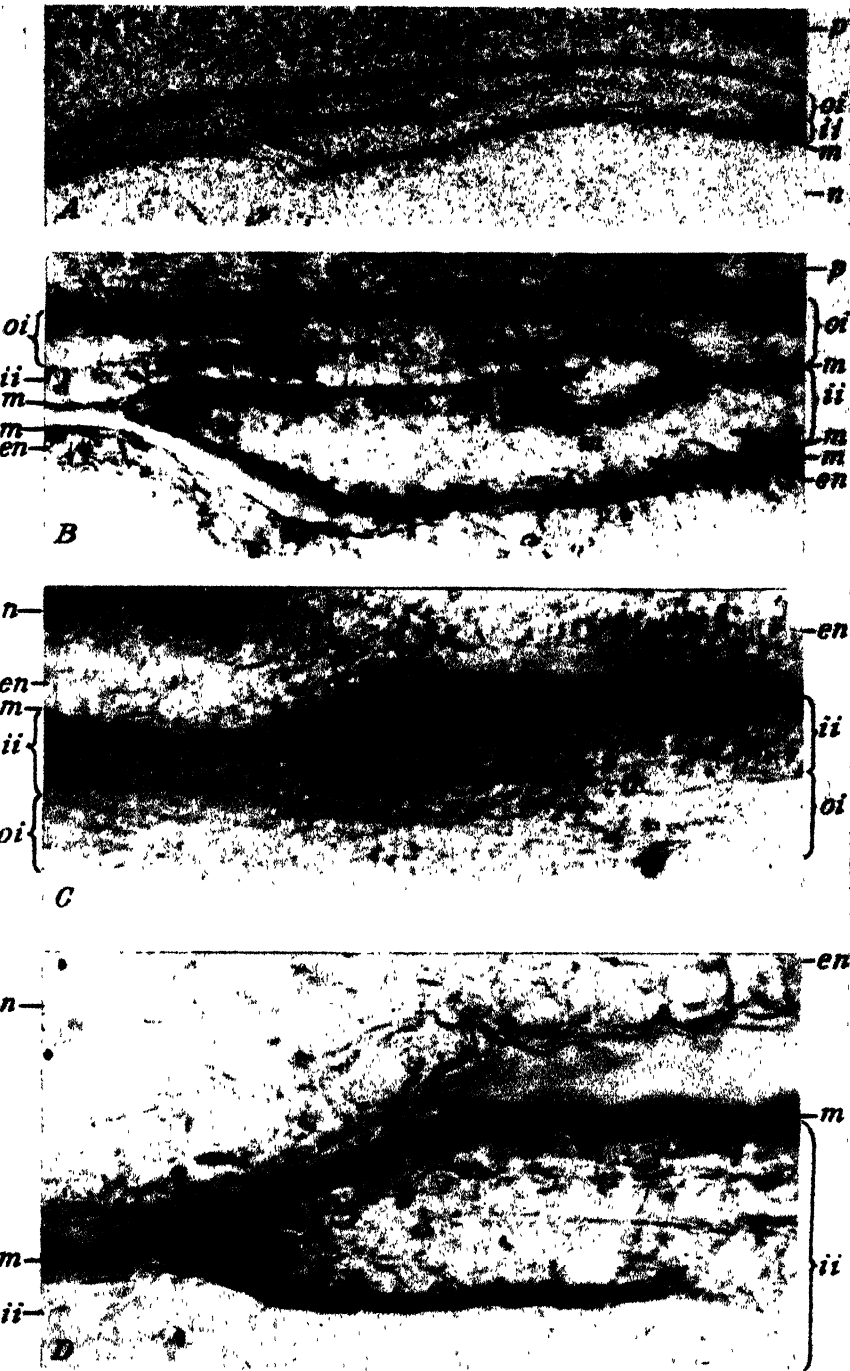
Folding of the inner integument along the sides of two young caryopses of inbred 176A. Freehand sections stained with Sudan IV, mounted in glycerin. A suberized membrane is present on the inner surface of the folded inner integument. *A* and *B*, Different magnifications of the same section; *B* shows the surface of the inner integument to be more highly suberized than the surface of the nucellus. Slight suberization of the outer surface of the inner integument near the base of the fold is also shown. *A*, × 85; *B*, × 210. *C* and *D*, Different magnifications of a section from another young caryopsis. A suberized membrane is present on the inner surface of the inner integument, none on the surface of the nucellus. In the folds observed, the apex pointed toward the germinal face of the caryopsis; the backward bend of the inner integument was toward the outer integument rather than toward the nucellus. *C*, × 280; *D*, × 590.

p, Pericarp; *oi*, outer integument; *ii*, inner integument; *m*, suberized membrane; *n*, nucellus; *en*, epidermis of nucellus.

after the disintegration of the intervening cells, by whatever thin patches of suberin may have been laid down on the outer surface of the inner integument (pls. 2, *B*, *C*; 7, *A*).

In those strains of corn in which folding of the inner integument has occurred, evidence of the integumental origin of a suberized layer appears to be conclusive. The membrane adheres to the inner surface of the inner integument, following its folds irrespective of their position with regard to the nucellus (pls. 6, *A–D*; 7, *B*, *C*).

The fact that pronounced folding of the inner integument occurs in some strains of corn has received little if any mention in studies of this problem. The probable explanation of its occurrence seems simple. The integuments are delicate structures, for the most part 2 to 4 cells thick, attached at the base of the ovule but otherwise distinct for a time in the narrow space between the spherical ovule and the concave inner surface of the ovary wall. The inner integument completely surrounds the ovule except at the micropyle; the outer integument does not completely cover the inner integument. During its development the inner integument must adjust itself to the curved space in which it is confined. If its growth is considerably more rapid or if it is continued longer than that of the neighboring parts, folding would seem inevitable.

Although such increased growth of the inner integument as is indicated by thickenings (pl. 7, *D*) or folds of various depths [7] may not be present in all cases, there seems to be no reason to believe that the origin of the primary membrane is essentially different in different strains of corn. Randolph studied varieties of dent corn as well as sugary and flint types, and stated (*11, p. 882*) that "no significant morphological differences were noted among the different types other than variations which might readily be attributed to differences in size, shape, or degree of maturity of the kernels."

SUMMARY

In the caryopsis of maize thin layers of suberin are formed on the surface of both the inner integument and the epidermis of the nucellus, the primary membrane being formed on the inner surface of the inner integument. The two membranes become physically united early in the development of the caryopsis and thereafter appear to be a single membrane common to both surfaces, much as is the case in barley. The membrane is thinner and more permeable on the germinal face of the kernel, a region where complete collapse of the cells of the inner integument first occurs.

In some strains of corn, areas on the outer surface of the inner integument also become lightly suberized. No instance of a complete coverage of the outer surface of the inner integument was observed, and no suberization of the outer integument was seen.

In young kernels in which folding of the inner integument occurs, the primary membrane is shown to adhere to the folded inner surface of the inner integument regardless of its position in relation to the nucellus.

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[7] Guérin (*3, p. 55*) may have observed a somewhat similar condition when he stated:
"L'ovule est toujours bitégumenté, et chaque tégument ne comporte, d'une façon générale, que deux assises cellulaires seulement. Parmi les genres que nous avons examinés, les *Zea*, *Tripsicum*, *Coix*, du groupe des Maydées, font exception à cette règle. Nous avons vu, en effet, que les téguments ovulaires peuvent, dans ces genres, comporter ensemble six et huit assises de cellules."

It appears, therefore, that maize conforms to the general pattern of the Gramineae in which the suberized membrane (or membranes) within the caryopsis is derived from one or both integuments. From the material examined in the present work it is concluded that the suberized semipermeable membrane in the corn kernel is derived primarily from the inner integument.

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APHID INFESTATION OF STRAINS OF CORN AS AN INDEX OF THEIR SUSCEPTIBILITY TO CORN BORER ATTACK [1]

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INTRODUCTION

The European corn borer (*Pyrausta nubilalis* (Hubn.)), a chewing insect, and the corn leaf aphid (*Aphis maidis* Fitch), a sucking insect, are both pests of corn (*Zea mays* L.). Differential susceptibility among corn strains to the corn borer has been recognized for several years. For corn-growing areas where the borer is a serious pest, the differential susceptibility to the insect among hybrid strains of corn provides a basis of major importance in the classification of breeding material. It is now known that there are similar differentials in susceptibility to the corn leaf aphid. The purpose of this paper is twofold: (1) To present data showing relations between the susceptibility of corn hybrids to aphid and to borer injury and, on the basis of these relations, (2) to suggest a new technique for evaluating susceptibility of hybrids to corn borer injury.

Burdensome techniques constitute the chief difficulty in evaluating susceptibility of corn strains to injury by larvae of the borer. The problem of sampling is a difficult one. In general, the choice has been between the difficult procedure of obtaining adequate samples of the stalks of the different strains under natural field infestation or of stalks that have been manually infested, and that of feeding etiolated leaves of the different strains to young larvae under laboratory conditions. Manual infestation reduces the number of plants needed for dissection but adds the requirements of rearing moths and applying their eggs to the strains under test. Further, the data from stalk dissections are subject to a large error unless the dissections are properly timed with reference to the usually different maturity of the strains under study. Laboratory feeding experiments also require the rearing of moths and the handling of eggs and larvae in addition to the necessity of providing a constantly fresh supply of food. Both methods may add an uncertainty of simulating natural conditions. A third choice, less often used, but satisfactory when populations are high and damage severe, has been to rate quantitatively the degree of larval damage to the leaves.

Since the inbred strains from which the corn hybrids are made are weak and consequently slow growing, they seldom carry sufficient natural populations of the corn borer to permit reasonably accurate selection. A simple technique, therefore, which can be used more advantageously on the inbred lines as such is needed.

REVIEW OF LITERATURE

Methods of studying strain differences in borer populations have been reported by Huber, Neiswander, and Salter;[2] Meyers, Huber, Neiswander, Richey, and Stringfield;[3] and Patch.[4] Correlations between borer and aphid populations on corn have been pointed out by Huber and Stringfield.[5] In the determination of strain susceptibility, all these investigators have relied upon natural field infestation, manual infestation, or both. The last method has been used extensively by Patch in studying the behavior of the borer under a wide range of conditions. Since 1937, Huber (unpublished data) has employed laboratory methods in which newly hatched larvae are fed etiolated portions of corn leaves from different strains for a period of 2 or 3 weeks.

MATERIALS AND METHODS

The strains of corn utilized in this study were developed or introduced by the cooperative corn project in Ohio and included inbred lines, commercial and experimental hybrids made from these lines, and the open-pollinated variety Woodburn.

In general, the material studied was from the regular breeding nurseries and field performance experiments. The inbred lines were in single-row or 4-row plots about 35 feet long. The number of replications was variable, depending upon how widely a given line was being used for seed increase and crosses. The hybrids were principally in regular field performance tests variously located in the State, and planted in 2- by 10-hill plots in fivefold replication or in 2- by 15-hill plots in fourfold replication within a modified Latin square design.[6]

ESTIMATING APHID SUSCEPTIBILITY

The degree of aphid susceptibility was estimated either by counting the number of stalks with aphid colonies on the newly emerged tassels or by counting the number of stalks determined to be barren because of heavy aphid population. Aphids begin to be noticeable just as the tassels emerge. For a period of several days, depending somewhat on weather and food conditions, the aphids remain in aggregations. As the tassels lose their succulence, the aphid colonies break up, and the individual aphids find food and shelter on other parts of the plant or are lost. After such dispersal, counts of infested tassels are not dependable. Observation has shown, however, that plants severely infested with aphids are commonly barren, or nearly so; hence the percentage of barren plants was taken as an estimate of aphid injury in experiments where tassel infestation was not obtained and where it was obvious that aphids had been an important cause of barrenness. In some tests, both measures were used.

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APHID RATING

In these studies, it has been necessary to take data in such plantings as were found to be infested rather than in systematically planned experiments, since it could not be known in advance which plantings would carry heavy insect infestations. Such data generally lack an element of orderliness, because some strains will be represented more or less often than others and in different plantings. The problem of compiling the data was met as follows:

(1) Since the inbred line Indiana WF9 was both highly susceptible and common to all plantings where inbred lines were studied, it was assigned a value of 100. Each other inbred line was assigned a rating in each planting by expressing its percentage of infested plants as a percentage of the corresponding infestation of line Indiana WF9.

(2) The hybrid Ohio K35 was also both highly susceptible and common to many different plantings. Accordingly, this hybrid was assigned a value of 100 and all other hybrids included in this study were rated in comparison with Ohio K35.

This procedure of comparing all strains to one which is used as a standard has its obvious weaknesses. It tends to make data more variable by introducing the variability of the standard. If, however, sets of such data from independent sources show definite relations to each other, the inference would be that there is a real relation, although its measurement is probably less precise.

PRESENTATION OF DATA

Table 1 presents a compilation of the data both on the inbred lines and on their single crosses, with the purpose of establishing an aphid susceptibility rating for the various lines. It will be noted that the number of tests involved ran from 1 to 18 for each line evaluated. Of the total of 18 tests, 15 were single-cross and 3 were inbred-line tests. Where single crosses were used, the value was the average of all the infestation counts where the line in question was one of the parents. Depending upon the degree of dominance and the susceptibility of the other parent lines, the single-cross ratings cannot be wholly comparable but are recorded as representing the best data available at this time.

TABLE 1.—*Relative aphid ratings of 30 inbred lines of corn, with Indiana WF9 assigned a value of 100 in each comparsion, 1938–40*

Inbred line	Number of tests	Relative rating	Inbred line	Number of tests	Relative rating
Ohio 02	6	201	Ohio 28	6	57
Iowa Cl 447	2	180	Ohio 17	3	55
Ohio 84	1	152	U. S. 187-2	8	51
U. S. 4-8	6	121	Iowa L289	3	51
Indiana Tr.	4	108	Illinois R4	4	50
U. S. 540	5	105	Indiana 38-11	5	49
Iowa Os426	3	101	Wisconsin CC5	2	34
Indiana WF9	18	100	Ohio 33	10	31
Iowa Os420	10	93	Ohio 51A	12	31
Ohio 26	10	93	Iowa I205	4	25
Illinois A	6	85	Ohio 07	2	25
Illinois 90	4	77	Ohio 40B	12	19
Ohio 56	3	74	Iowa L317	7	18
Ohio 65	4	72	Ohio 67	3	14
Illinois Hy	9	68	Ohio 51	4	9

Table 2 shows the predicted and observed ratings for aphid susceptibility of a group of double crosses included in the cooperative corn performance tests. The ratings of the four parent lines of each double cross are averaged and are given in the fourth column as the predicted rating for the double cross. The observed ratings in the fifth column represent the mean of the relative ratings of the aphid infestation on each planting with Ohio K35 used as a standard of comparison in each test. The correlation coefficient between the predicted and observed aphid ratings, as expressed by the values in table 2, is 0.53, a significant correlation.

TABLE 2.—*The predicted aphid rating of 28 double crosses on the basis of aphid ratings of parental inbred lines and the actual percentage of stalks of double-cross hybrids infested with aphids or that were barren because of aphids, in 16 cooperative test plantings, 1938–40*

Hybrid	Pedigree	Number of tests	Predicted rating	Actual mean aphid rating
Ohio 465	(56 × Os420) (65 × 02)	2	110	54
Ohio K35	(65 × 02) (Hy × 26)	16	109	100
Iowa 931	(L289 × C1 447) (Os 420 × Os426)	4	106	75
Ohio 38	(WF9 × Hy) (40B × 02)	6	97	109
Indiana 608B	(A × Tr) (WF9 × Hy)	2	90	122
Ohio W17	(56 × 4–8) (51 × 84)	16	89	74
Ohio 36	(51A × WF9) (40B × 02)	1	88	83
U. S. 44	(187–2 × 4–8) (Hy × 540)	3	86	88
Indiana 425	(A × 90) (WF9 × Hy)	3	83	119
Ohio K23	(26 × 51) (65 × 84)	7	82	54
Illinois 172	(R4 × Hy) (A × 540)	3	82	32
U. S. 52	(Hy × 67) (4–8 × 540)	5	77	118
Illinois 384	(A × Hy) (WF9 × R4)	5	76	56
U. S. 65	(51 × 4–8) (Hy × 540)	15	76	58
Ohio 563	(WF9 × Os420) (51A × Hy)	1	73	37
Ohio 387	(WF9 × 38–11) (51 × 4–8)	2	70	78
Ohio 160	(Os420 × Os426) (51 × 56)	1	69	6
Iowa 939	(L289 × I205) (Os420 × Os426)	16	68	68
Indiana 614	(A × Hy) (R4 × Tr)	2	68	37
Ohio 26	(WF9 × Os420) (33 × 40B)	3	61	97
Ohio M15	(51 × 26) (A × CC5)	2	55	40
Ohio 54	(WF9 × 40B) (51A × Hy)	3	55	52
Ohio 418	(40B × 4–8) (51 × 65)	4	55	10
Ohio 398	(51 × 4–8) (Hy × L317)	2	54	35
Ohio 487	(51 × 56) (Os420 × 40B)	1	49	0
Ohio 30	(WF9 × 07) (33 × 40B)	3	44	45
Ohio C14	(51 × 56) (67 × Hy)	9	41	54
Ohio 32	(28 × 187–2) (33 × 40B)	6	40	48

Table 3 gives the corn borer population for a group of hybrids grown at Holgate, Ohio, in 1936, on which the population counts were taken in July; and the expected aphid rating was taken from values in table 1. The correlation coefficient between the number of borers per stalk, column 2, and the estimated aphid rating, column 3, is 0.74, a significant value.

One of the experimental plantings for comparing the field performance of single crosses in 1939 was located at Van Wert, Ohio. The 100 strains included in this test ranged from an average of 1 to an average of 67 percent of the stalks infested with aphids, and from 0 to 18 percent of the stalks were barren and heavily infested with aphids. Corn borer populations varied from 2.5 to 6.8 borers per stalk. Additional data taken on these plots included counts of borer holes in unit areas of the stalk as a measure of borer population, midsilking date as a measure of relative maturity, and stalk height at the time of moth flight as a partial measure of egg deposition.

The partial correlation of aphid infestation and borer holes per stalk with height and silking taken into account was 0.29, a significant value.

TABLE 3.— *Borer population per stalk and estimated aphid rating for a group of single, three-way, and double crosses, Holgate, Ohio, 1936*

Hybrid	Pedigree	Borer population per stalk	Estimated aphid rating
Iowa 931	(L289 × C1 447) (Os420 × Os426)	2.2	106
Iowa 939	(L289 × 1205) (Os420 × Os426)	2.2	68
	(540 × 67) × L317	1 7	46
	(65 × 51) (17 × 26)	1 7	57
	(56 × 67) × L317	1.5	35
	(Tr × 67) × L317	1 4	47
Ohio W17	(51 × 84) (56 × 4-8)	1.5	89
	(Hy × 67) × L317	.9	33
	(R4 × 67) × L317	.8	27
	(540 × Hy) (67 × L317)	9	51
	(67 × L317)	.7	16

A similar comparison involving 70 strains, mostly single crosses, at Van Wert in 1940, carried a borer population averaging nearly 12 borers per stalk and an aphid infestation up to 24 percent. With this high borer population, strain differentials in leaf and stalk damage in late July were conspicuous and measurable. Because it was not possible to obtain the borer population for each individual strain, each strain was rated as to the relative amount of damage to the stalks and leaves. Strains with almost no leaf punctures or very few (fig. 1) were rated 0, whereas those showing holes and ragged leaf punctures (fig. 2) in practically every leaf, together with a majority of leaf midribs broken, were classified in the highest category, viz, 4. Strains with an intermediate amount of damage were placed in intermediate categories. Since there is a significant relation between such measurements and borer population and since leaf-damage measurement is more rapid, it was used as a measure of larval population. The correlation of this value with the percentage of stalks showing aphid infestation was 0.44, a significant value. The same measurement of leaf and stalk damage showed a correlation of 0.40 with the number of borer holes per stalk. This correlation is also significant.

On 19 strains, including single-cross, three-way, and double-cross hybrids planted at Van Wert in 1940, data were taken on the number of eggs and borers per stalk. The average egg masses per stalk on July 13 ranged from 1 to 2.4 on the different strains, and the borer population in early August, from 10.2 to 18.5 per stalk. Aphid infestation on these plots was negligible. However, the predicted aphid ratings of these strains showed a partial correlation of 0.83 with borer population when the number of egg masses per stalk was taken into account.

Additional evidence of the relationship between borer and aphid populations is shown in data taken from 33 inbred lines planted for comparison in fourfold replications of 4- by 10-hill plots at Van Wert in 1940. Egg counts were made and borer population was determined on only 16 of these 33 strains. The partial correlation of borer population and the percentage of stalks infested with aphids,
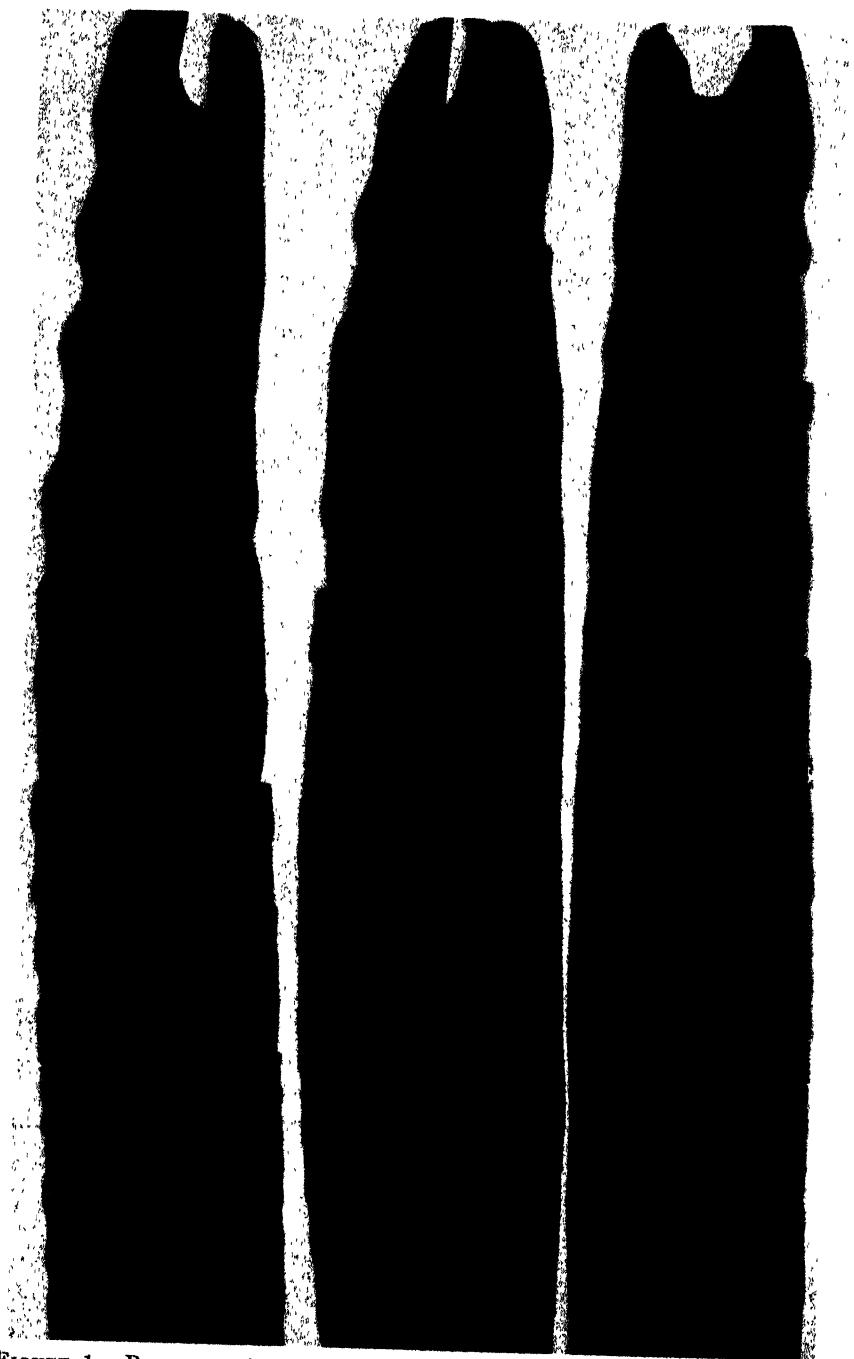
FIGURE 1.—Representative leaves of a corn strain rated in the 0 category of damage by corn borer larvae.
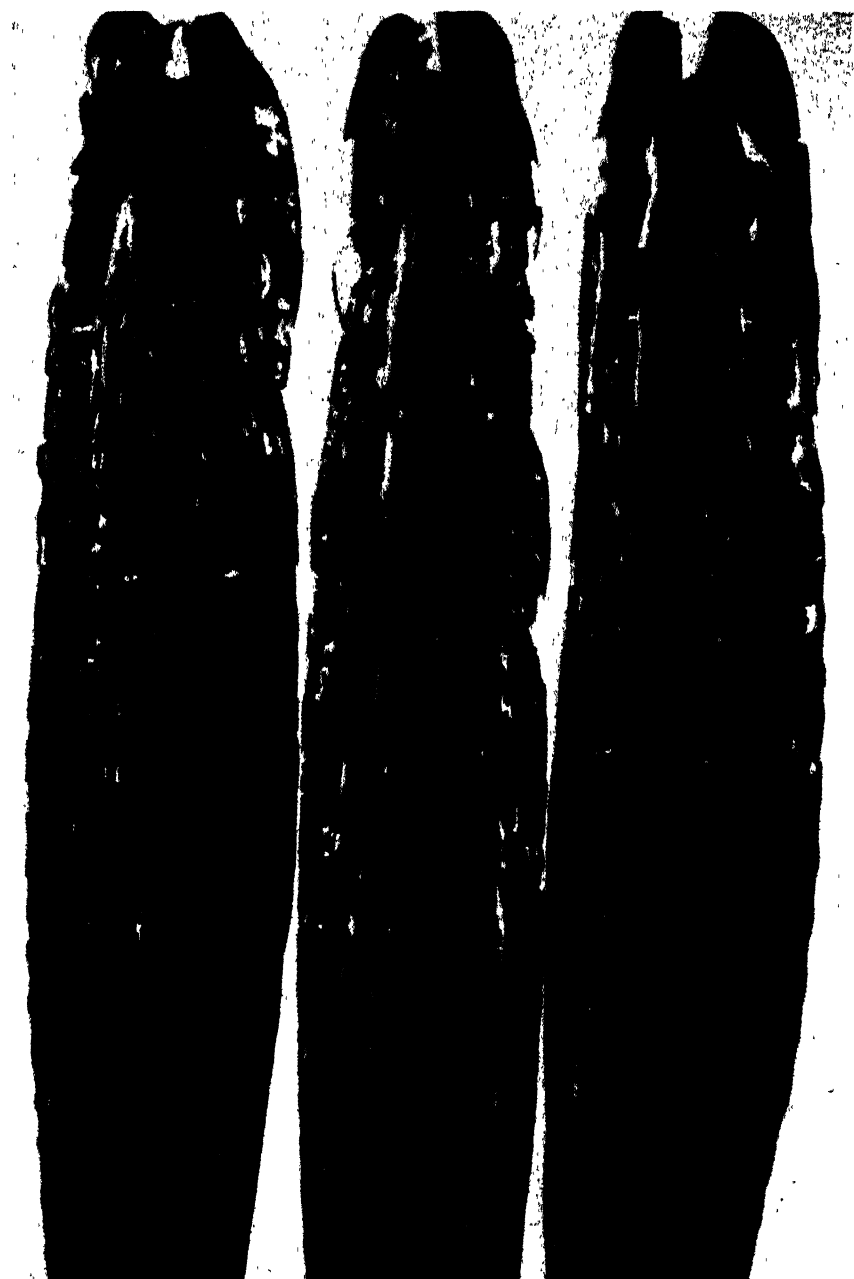
FIGURE 2.—Representative leaves of a corn strain rated in category 4 of damage by corn borer larvae.

with eggs taken into account, was 0.50, a significant correlation. When borer holes per stalk were used as a measure of borer population on the entire planting of 33 strains, the correlation with the percentage of aphid-infested stalks was 0.37. This also is a significant value.

DISCUSSION

The ratings on aphid susceptibility of inbred lines as indicated in table 1 are obviously subject to considerable error. As further data are accumulated, these values will fluctuate. For example, inbred line Ohio 84 is rated on the basis of one test only so far as its comparison with Indiana WF9 is concerned. The level of aphid infestation in the various comparisons involved ranged from a maximum of 9 percent in 1 planting to 95 percent in another, with an average of about 35 percent for the total of 18 plantings.

The ratings of the double crosses in table 2 are also subject to error. The total of 28 hybrids include 4 with only 1 test. Of these 4 hybrids, 1 is new and the other 3 were discarded as commercially undesirable after the first test. Where the percentage of stalks infested was used as the basis of aphid rating on the double crosses, the maximum infestation on 12 plantings ranged from 12.4 to 67.3 percent. On 4 of the double-cross tests where the percentage of barren stalks was used as a measure of aphids, the percentage barren ranged from 3.8 to 8.3. A further source of error in rating double crosses may be a considerable degree of dominance at either the high or low end of the susceptibility scale. Moreover, the ratings are based on data assembled from fields in various locations subjected to widely different environmental conditions, factors which in themselves are likely to introduce variability in the relative ratings.

With these considerations in mind, the correlation coefficient of 0.53 between the predicted and observed aphid ratings of the double crosses (columns 4 and 5, table 2) is interpreted as being reasonably high. It indicates that the relative susceptibility to aphid injury of inbred lines is transmitted to their hybrid progenies in a measurable degree, and that knowing the susceptibility of inbred lines, one can reasonably make predictions as to the susceptibility of their crosses.

The purpose of this paper, however, is not primarily to demonstrate the inheritance of susceptibility to aphids, but rather to use the facts involved to build hybrids less susceptible to the corn borer. The problem is to demonstrate the relation, if any, between the behavior of the aphid and the borer on the same strains of corn. Evidence of a positive relation is indicated in the partial correlation of 0.29 between borer holes per stalk with height and silking taken into account. Although this correlation is low, it is significant. In connection with this particular test, there was evidence of considerable competition between aphids and borers. Aphids began to draw heavily on the plant nutrients earlier than the borers. As the injured plant is starved, it differentiates more slowly and becomes the type of plant that has long been known to provide a poor medium for borer development.

Further evidence of a relation between the susceptibility of corn to aphid and to borer injury appears in the correlation of 0.44 between leaf damage as caused by the borers while still in the first to third instars and the percentage of aphid-infested stalks. The partial

correlations of 0.83 between actual borer populations per stalk and predicted aphids with egg masses taken into account, and of 0.50 between borer population and the percentage of aphid-infested stalks with eggs taken into account, together with the correlation of 0.74 between borer population and predicted aphids at Holgate, all indicate that strains preferred by the aphid are in general also preferred by the borer. Further evidence of this relation is the correlation of 0.37 between borer holes and the percentage of stalks infested with aphids. It is not within the province of this discussion to suggest the possible factors involved, but it does seem that the relationship is not only unique but also that it can be made to serve a useful purpose. It would appear that the information at hand can be used to build hybrids not only less susceptible to the aphid but at the same time less susceptible to the corn borer.

SUMMARY AND CONCLUSIONS

Data are presented to show (1) that inbred lines of corn and their hybrids exhibit heritable differences in susceptibility to the corn leaf aphid, and (2) that susceptibility to the corn leaf aphid, a sucking insect, was measurably correlated with susceptibility to the corn borer, a chewing insect. It would seem that at least preliminary classifications of breeding material in respect to susceptibility to the corn borer might be made on the basis of aphid populations. Aphid infestations can be estimated rapidly, whereas corn borer populations must be estimated by slow and burdensome methods.

AN IMPROVED METHOD FOR DETERMINING THE DISTRIBUTION OF SALT AND WATER IN CURED HAMS [1]

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INTRODUCTION

Of fundamental importance in the production of cured hams is a knowledge of the action of the curing ingredients within the meat. The composition of curing mixtures and their method of application to hams have been the subject of study by several investigators (*10, 13,22*).[2] In most of this work, however, results have been expressed in terms of palatability and chemical analyses based on the entire ham and have contributed little information on the distribution, within the meat itself, of the curing ingredients. Other workers (*2, 16, 18*) have shown that different portions of cured hams vary in their content of curing ingredients, and that curing and aging procedures affect the distribution of salt in hams (*11, 17, 20, 24*).

Callow (*6, 7, 8*) has reported rates of salt and water absorption and dextrose penetration into pork muscle or parts of muscle. These papers, together with the works of Richardson (*23*) and Brooks (*5*) on the effect of nitrate and oxygen on the color of cured pork, have been reviewed and interpreted by Jones (*14*). However, these studies were conducted usually as in vitro experiments; consequently, there is no assurance that the results apply equally to meat within the entire ham. Variations in the composition of the different ham muscles, together with their relative location within the ham, may influence processing results. Table 1 gives determinations by the present authors of the ether-extract, water, and crude-protein content of the ham muscles arranged in the order of their ether-extract content. The importance of a knowledge of the quantity of fat and connective tissue in pork and their relation to the rate of salt penetration has recently been demonstrated by Banfield and Callow (*1*) as well as by Clarenburg (*9*).

Since salt [3] is universally used as the principal preservative in cured hams, the role played by this ingredient during the processing procedure is of particular importance. A ham, however, is not sufficiently homogeneous to permit accuracy in random sampling unless the material has been thoroughly ground. Grinding an entire ham, on the other hand, mixes its component parts, and samples from such material fail to provide information on the distribution of the curing ingredients throughout the ham. Consequently, in studies to determine the factors responsible for the rate of salt absorption, entire hams have usually been processed, and selected parts have served as the sources of sampling material.

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TABLE 1.—*Ether-extract, water, and crude-protein content of 10 muscles dissected from a 13-pound fresh ham*

Muscle	Ether extract	Water	Crude protein [1]
	Percent	*Percent*	*Percent*
Vastus lateralis	3 37	77. 32	94. 36
Adductor	4. 47	75. 02	92. 94
Semimembranosus	5 34	74. 13	92. 35
Rectus femoris	5. 66	75 51	92 13
Sartorius	6. 54	74. 00	91 77
Vastus medialis	7. 05	73. 70	93 25
Gracilis	7. 10	72 29	92 35
Biceps femoris	7 11	74 36	94 73
Semitendinosus	8. 50	73. 38	92. 35
Vastus intermedius	10 95	71 54	89 64

[1] Determined on fat- and moisture-free basis

In studies in which the entire ham provides the source of material for investigation, a proper sampling technique is essential to the solution of the problem. The ideal sample is one from which a knowledge of the quantity and distribution of any constituent in the ham can be obtained. Consequently, a study was made with the object of developing a method of sampling cured ham that would give detailed information on salt distribution and on water distribution as well, since the latter may be valuable in explaining certain salt movements throughout the meat, particularly those accompanying the process of aging. The technique of such a method was suggested in 1936, as reported by Mohler (*19*), and has recently been applied successfully to cured lamb legs by Besley and Hiner (*3, 4*). The present paper gives some of the detailed analytical data that prompted the use of this method.

PREVIOUS METHODS FOR DETERMINING THE DISTRIBUTION OF SALT

As early as 1911 McBryde (*16*), in a study involving sour and sound hams, attempted to determine the relative distribution of salt and potassium nitrate in hams. His sampling technique provides for a cross section about 2½ inches wide cut from the center of the ham at right angles to the femur. The ham is trimmed along the lines *lm* and *no*, as shown in figure 1, and four slices (*A, B, C,* and *D*) of approximately equal thickness and parallel to the face of the ham are removed. Slice *A* includes the face of the ham, slice *B* the bone (femur), slice *C* the meat between the bone and fat of the skin side, and slice *D* practically all fat.

Another method for determining salt distribution in hams was proposed by Lewis and outlined by Moulton (*21, pp. 408–409*). According to this method, a 1-inch cross-section slice is taken from the ham directly behind the aitchbone, and from the center of this slice a strip extending from the face to the skin and adjacent to the femur is removed and divided into five horizontal sections, as shown in figure 2. Using this method, Moulton presents data (*20*) indicating the salt distribution in partly cured and fully cured hams, before and after smoking. Barnicoat (*2*) and, more recently, Miller and Ziegler (*17, 18*) and Ziegler and Miller (*24*) used a similar sampling technique. Grimes, Sewell, and Cottier (*11*) based their work on three divisions

FIGURE 1.   Location of samples in cross-section slice of ham according to the sampling technique of McBryde (*16*).

of a cored sample taken crosswise through the center of the ham from the flesh side to the skin side.   Apparently all these investigators used the sampling method originally suggested by Lewis or some modification of it.
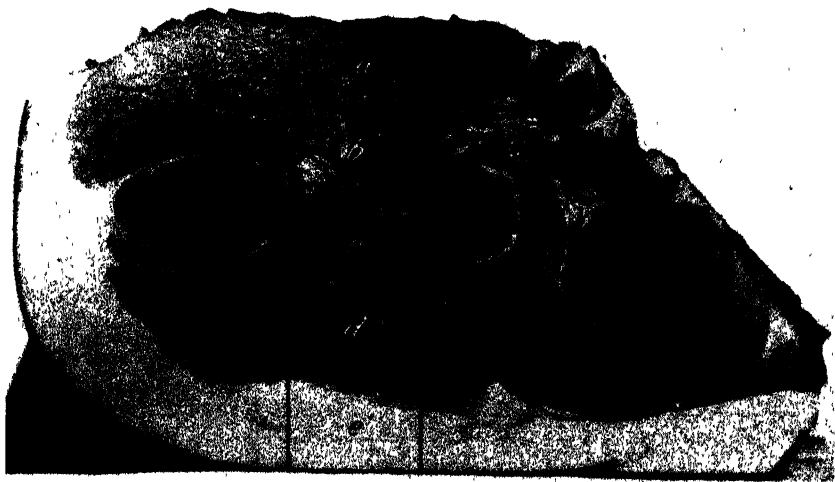
FIGURE 2.—Location of samples *a* to *e* in cross-section slice of ham cut according to the sampling technique of Moulton (*21*) and others.

PRELIMINARY STUDIES

To determine the value of these sampling techniques the following study was made. Six representative hams were removed from a batch of dry-cured hams after 46 days of curing. These hams had been cured with a mixture consisting of 8 pounds of salt, 2 pounds of sugar, and 2 ounces of saltpeter for every 100 pounds of meat. This mixture had been divided into three applications during the first 15 days of curing. Two of these hams were analyzed immediately after removal from cure, two were smoked, and two were smoked and then stored for a period of 3 months. The smoke treatment consisted of 20 hours of hardwood smoking at 110° F. Storage took place in a ham room in which the temperature fluctuated with surrounding

FIGURE 3.—Location of samples in cross-section slice of ham according to the sampling technique used in preliminary work by the authors.

conditions and averaged about 55° for the 3-month period. Each ham was sampled according to the technique used by McBryde (*16*), slice *D* (fig. 1) being omitted, however, since it contained little lean meat. Each of the three slices was then divided into four parts, which resulted in a grid arrangement of samples as shown in figure 3. By such an arrangement the features of the technique proposed by McBryde were combined with those proposed by Moulton (*21*), the latter method being represented by the *b* segments in figure 3, but consisting of only three instead of the four segments shown in figure 2. Barnicoat (*2*) and Miller and Ziegler (*18*) likewise omitted the fat sample from their data for the same reason that slice *D* was omitted in the present study.

After the removal of adhering fat and bone, the segments shown in figure 3 were finely ground and aliquot portions examined for their salt content by the second method suggested by Kerr (*15*) for the deter-

mination of salt in meat.   In the unsmoked cured hams and those out of smoke, segments *a* and *b* were combined, as were segments *c* and *d*, in each of the three slices of the cross-section slice.   In those stored for 3 months each sample was analyzed separately.   The results obtained in this study are shown in table 2.

TABLE 2.—*Average salt distribution in hams after the curing, smoking, and aging processes, as determined by the McBryde (16) method of sampling, with a further division of each slice into four parts*

[All hams cured for 16 days]

Number of hams	Average weight of hams—		Stage of processing	Segments of slice [1]	Salt [2] in indicated segments of slice--			
	Before any processing	After indicated processing			*A*	*B*	*C*	Total
	Pounds	*Pounds*			*Percent*	*Percent*	*Percent*	*Percent*
2	14.7	14.3	46 days in cure	{ *a* and *b*	7.99	3.01	1.50	4.48
				{ *c* and *d*	8.81	4.62	2.85	5.59
				Total	8.40	3.80	2.25	5.04
2	16.1	14.6	Out of smoke	{ *a* and *b*	7.31	3.59	2.21	3.89
				{ *c* and *d*	7.43	6.45	5.48	6.45
				Total	7.37	5.09	3.41	5.06
2	15.3	12.6	3 months of storage.	{ *a*	4.99	4.34	3.23	4.51
				{ *b*	5.96	4.23	4.73	4.87
				{ *c*	6.47	5.04	6.30	6.12
				{ *d*	3.90	8.24	7.40	6.80
				Total	5.35	5.34	5.36	5.35

[1] See figure 1.
[2] Wet basis.

The data obtained by the McBryde sampling technique (fig. 1) indicate that as a result of the curing process slice *A* contained considerably more salt than slice *B* and still more than slice *C*.   A better distribution followed smoking, and at the end of 3 months of storage apparently an even distribution had been acquired.   On the other hand, when each of the three slices was divided into four parts (fig. 3), the combined segments *a* and *b* consistently had less salt than the combined segments *c* and *d* at all stages of processing.   Thus, the entire cross section of the hams, when sampled longitudinally by one method, had a different salt distribution than when sampled perpendicularly by another method.   Therefore, the division, into 12 samples, of the material used in the McBryde technique merely emphasized to a still greater degree the lack of uniformity in salt distribution. Furthermore, neither the limits of this salt distribution nor the total salt content of the entire ham cross-section slice appeared to be adequately indicated by the *b* segments, the sampling material suggested by Moulton.

Miller and Ziegler (17, 18) pointed out that equalization of salt distribution in cured hams does not become apparent until a storage period of at least 30 days has elapsed.   The results in table 2 indicate that a storage period of 3 months failed to produce an equal distribution in all portions of cured hams.   In further tests, several entire muscles were removed from a cured ham that had been kept at 107° F. for 20 weeks and stored for a period of 3½ years, and their salt and

moisture content determined. Results of these analyses are shown in table 3. Here, again, appears evidence of the uneven distribution of salt in hams aged for a long period. A study of tables 1 and 3 shows that the vastus intermedius muscle, which contains the most fat, contains the least salt and that the vastus lateralis muscle, which contains the least fat, is among the higher salt-containing muscles as a result of the long aging period. Although this analogy does not hold for all the muscles included in the table, it does raise again the question of the relationship between salt absorption and the chemical composition of the muscle tissue.

TABLE 3.—*Average salt and water content of nine muscles from a ham stored in ham room for 3½ years*

Muscle	Salt [1]	Water	Muscle	Salt [1]	Water
	Percent	*Percent*		*Percent*	*Percent*
Vastus intermedius	7.58	24.15	Semitendinosus	12.33	28.23
Vastus medialis	10.34	22.83	Semimembranosus	12.71	19.58
Adductor	10.35	24.00	Vastus lateralis	13.58	25.36
Rectus femoris	11.39	29.66	Gracilis	13.95	19.86
Biceps femoris	11.85	26.10			

[1] Wet basis.

STUDIES INVOLVING AN IMPROVED SAMPLING TECHNIQUE

The results of the preliminary studies indicated that neither the McBryde (*16*) technique nor that suggested by Moulton (*21*) provided sample material from which a comprehensive knowledge of the salt distribution in cured hams could be obtained. For such a study the modification of these sampling methods, as shown in figure 3, appeared to be an improvement. However, the fact that all hams are not shaped or trimmed alike makes it difficult to obtain exactly comparable samples, particularly when the method of obtaining them is based on measurements related to such variables as the size and shape of the ham. Furthermore, since variations in the composition of individual muscles may influence their ability to absorb salt as well as to lose moisture, it is desirable to distinguish between muscles in sampling. Therefore, muscle segments in the cross-section slice of the ham, as shown in figure 4, were used for sample material. The fact that in such a method the location of samples is related directly to the anatomy of the ham makes it easier to specify the location and permits a comparison of hams with assurance that the samples are from the same regions.

EXPERIMENTAL PROCEDURE

The specific technique of the proposed method of sampling is as follows. A cross-section slice is cut from the ham not more than 2 inches below the aitchbone and at right angles to the femur. The thickness of this slice is determined by the size of the ham and should be approximately one-fifth of the distance between the aitchbone and the stifle joint along the femur. The muscle segments in this slice, when carefully dissected from adhering fat and connective tissue, constitute the material for analysis. Each of these segments is cut into fine pieces with a pair of scissors, placed on tared watch glasses, and the moisture determined after heating in a drying oven

for 16 hours at 214° F. The dried material is then finely ground in a mortar and a representative sample removed for salt determination, the second method suggested by Kerr (*15*) being used. Since dried material is used for salt analyses, greater concentrations of silver nitrate and thiocyanate solutions than are recommended in the Kerr wet procedure may be necessary. The strength of these solutions depends on the quantity of the dried sample used, as well as on its relative salt content.

As a result of fat differences and various processing treatments, the moisture content of ham muscles varies slightly, thus making it necessary to express salt results on a dry basis for accurate interpretations.

Cutting up the muscle segments with scissors prior to desiccation

FIGURE 4 —Location of muscle segments in cross-section slice of ham according to proposed sampling technique: *a*, Semimembranosus; *b*, adductor; *c*, sartorius; *d*, vastus medialis; *e*, semitendinosus; *f*, biceps femoris; *g*, vastus intermedius; *h*, rectus femoris; *i*, vastus lateralis.

is a substitute for the usual procedure of passing the material through a meat grinder. Not only does this eliminate a somewhat laborious operation, considering the number of muscle segments used, but it results in a better mixing of the fat and lean for uniform aliquot sampling. Grinding the dried muscle segments in a mortar and pestle usually produces a well comminuted material, as the fat mixes with the lean in much the same manner as shortening is kneaded into flour.

APPLICATION OF PROPOSED METHOD TO HAMS OF VARIOUS CURES

Hams that had been cured 5, 15, and 30 days in a dry- and a brine-curing process were sampled in accordance with the proposed method.

For the dry-curing process, a mixture of 8 pounds of salt, 2 pounds of brown sugar, and 2 ounces of saltpeter per 100 pounds of meat

was used. One-half of this mixture was applied at the beginning, one-half of the remainder at the first repacking 5 days later, and the rest at the end of 15 days' curing, when the hams were repacked again. For the brine cure the same proportions of curing ingredients dissolved in 4½ gallons of water were used. The hams to be cured were covered with the brine and repacked after 5 and 15 days in the curing solution. All hams were cured at 38° F.

Several cross-section slices from each ham were obtained, as shown in figure 5, for the purpose of determining the most representative slice to be found in that portion of the ham located between the aitch-bone and the stifle joint. This is the area most commonly selected for sampling material and is considered to be fairly representative of the entire ham.

Salt and water determinations were made on those muscle segments in each slice from which sufficient material was available for analysis. Five slices were obtained from the hams cured 5 days, but the lack of sufficient muscle segments in the fifth slice resulted in the use of only four slices for sampling material in the remaining hams. The results are shown in table 4.

FIGURE 5.—Location of cross-section slices 1 to 5 in hams according to proposed sampling technique.

TABLE 4.—*Salt[1] and water content of muscles in cross-section slices of hams dry- or brine-cured for various periods*

[1 ham for each method and period of cure]

Weight of ham in pounds— Uncured	Weight of ham in pounds— Cured	Method of cure	Period of cure	Cross-section slice No.[2]	Adductor Salt	Adductor Water	Biceps femoris Salt	Biceps femoris Water	Gracilis Salt	Gracilis Water	Rectus femoris Salt	Rectus femoris Water	Sartorius Salt	Sartorius Water	Semimembranosus Salt	Semimembranosus Water	Semitendinosus Salt	Semitendinosus Water	Vastus intermedius Salt	Vastus intermedius Water	Vastus lateralis Salt	Vastus lateralis Water	Vastus medialis Salt	Vastus medialis Water	Average total slice Salt	Average total slice Water

(Numeric data table — columns and rows as printed; see figure for detailed values.)

[1] Dry basis.
[2] See fig. 5.

A considerable amount of variation in salt content was found between muscle segments in the same slice, differences in water content being less marked. However, the data were obtained during the periods of maximum salt absorption in the hams, when differences in the composition and location of muscles would have a pronounced effect on salt distribution. Between cross-section slices 1 and 5 in the same ham, some muscle segments, such as the biceps femoris and rectus femoris, increased in salt content and decreased in water content rather consistently, whereas others, including the semimembranosus, gracilis, and adductor, usually lost salt and gained water. The relation of the quantity of each muscle to the total quantity of muscle examined in each cross-section slice from the hams included in table 4 is shown in table 5. Since in any one slice the biceps femoris and rectus femoris together contribute less to this total than the semimembranosus, gracilis, and adductor segments, this larger proportion of the group of muscles having a consistent loss of salt may explain the apparent decrease in salt between cross-section slices 1 and 4. The gracilis muscle appears to take up salt rapidly as the curing period lengthens, and the absence of this muscle segment from cross-section slice 1 lessens the quantity of salt in this slice. Although this muscle represents only 6.4 percent of the total muscle examined (table 5), in the earlier stages of curing it contains 30 to 40 percent of the total quantity of salt in the slices.

TABLE 5.—*Relation of each muscle segment to the average total quantity of muscle in each cross-section slice from hams included in table 4*

| Muscle segment | Proportion of indicated muscle segment in cross-section slice No. | | | | | |
	1	2	3	4	5	Average total
	Percent	Percent	Percent	Percent	Percent	Percent
Semimembranosus	25 0	27. 2	23 4	21 8	33 2	25 0
Biceps femoris	18 2	17. 1	17. 0	21 8	28 6	18 8
Semitendinosus	11 7	9. 9	8 9	12 4	17 4	11 0
Rectus femoris	10 7	11 6	12 6	7 8		10 3
Vastus lateralis	10 9	8. 6	10. 1	5 0		8. 3
Vastus intermedius	9. 3	8. 4	7. 6	5 2		7. 3
Vastus medialis	4. 4	5 9	8. 7	11 6		7 1
Gracilis		4 2	6 5	12 9	19 6	6. 4
Adductor	7 8	5 0	3 2			3 9
Sartorius	2. 0	2 1	2 0	1 5	1 2	1 9

The relation between the quantity of muscle in each cross-section slice and that in the entire portion analyzed between the aitchbone and stifle joint was determined. That a greater proportion was found in the first three slices may be seen from table 6. When five slices composed the sampling material, practically 70 percent was represented by slices 1, 2, and 3. When four slices were used the quantity of muscle in the first three slices was slightly more than 80 percent of the total sample. Furthermore the adductor muscle, which is attached to the aitchbone, does not extend sufficiently along the femur to be included in either the fourth or fifth slice. Since Hiner and Hankins (*12*) have shown this muscle to represent approximately 5 percent of all the separable lean in hams, it would appear that it should be included in any representative sampling method. The same state-

ment applies to the gracilis muscle, whose segment does not appear in cross-section slice 1. This is a thin surface muscle that covers a large portion of the face of the ham and was trimmed away close to the aitchbone in these hams.

From the evidence presented it appears that the choice of cross-section slices most representative of the ham and suitable for use in the proposed sampling technique would be one of the first three, provided all slices have the gracilis muscle. The authors selected slice 2 (fig. 5) for the reason that it usually represents the thickest and widest part of the ham and therefore surpasses other slices in total weight of lean meat. Furthermore, it contains representative segments of all the muscles between the aitchbone and stifle joint and has a salt and water content similar to that of the average slice.

TABLE 6.—*Relation of the quantity of meat in each cross-section slice to the total quantity sampled from each ham*

[1 ham for each method and period of cure]

Method of cure	Period of cure	Proportion of meat in cross-section slice [1] No —				
		1	2	3	4	5
	Days	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Dry	5	21 1	26. 5	23 6	21. 7	7 1
Brine	5	21. 8	23. 8	22. 8	21 4	10 2
Dry	15	22 4	28 8	27. 7	21 1	....
Brine	15	34 7	28 1	24. 2	13 0	....
Do	30	24 1	29. 8	25. 4	20. 7	....
Average		23 5	26 7	24. 3	20 4	5 1

[1] See fig. 5.

DISCUSSION

The wide differences in salt content between muscle segments in the same cross-section slice indicate the necessity of including as many of these muscles as possible in the sample to be used in determining the salt distribution in cured hams. A study of figures 2 and 4 shows that only portions of a few of these muscles are included in the sample material proposed by Moulton (*21*). These muscles are the adductor, semimembranosus, and biceps femoris, with the gracilis muscle making a fourth one if it is not trimmed away as was the case in slice 1. Although table 5 shows that these muscle segments represent more than 50 percent of the meat analyzed in any cross-section slice, only portions of these segments are included in the sample used in Moulton's method. Actually, in the hams analyzed after 3 months of storage, more than two-thirds of the total meat, which contained 70 percent of the salt, was found to be outside of the sample material used in Moulton's technique (*21*).

Although the ham-sampling method for salt determination used by McBryde (*16*) provides more of the cross-section slice for analysis, it does not take into account the fact that individual muscles located equally distant from the face of the ham may vary widely in salt content. Such differences are not distinguishable when the whole segment is analyzed as one sample. It may be argued, however, that from the standpoint of sampling, McBryde's main interest was to show that sour and sound hams did not differ in chemical analysis, and salt distribution, therefore, was not the primary part of his studies. In

this connection, the present authors applied their proposed sampling technique to several sour and sound hams that had received identical curing and smoking treatments. Their findings agreed with those of McBryde in that there was a similarity in the salt content of sour and sound hams. In addition, however, the authors' data showed that the biceps femoris and the vastus intermedius muscles contained such small quantities of salt as to exert little, if any, bactericidal action in these regions where spoilage seemed to be concentrated, despite the fact that adjoining muscle segments of equal or further distance from the face of the ham appeared to have two to three times as much salt. These results suggest that the failure of salt to penetrate in sufficient quantity into certain muscles in hams may be largely responsible for the development of spoilage in these areas, provided the spoilage organisms are present. It is also felt that McBryde would have attached more significance to ham spoilage resulting from a lack of penetration of the preserving fluids had he used a sampling method that showed more details of the salt distribution.

SUMMARY

This paper describes a method of sampling cured ham by which it is possible to obtain detailed information on salt (sodium chloride) distribution and also on water distribution. A knowledge of the latter may be valuable in explaining certain salt movements throughout the meat, particularly those accompanying the process of aging.

The results of a preliminary investigation revealed that the sampling techniques of several other investigators did not provide material from which a comprehensive knowledge of the salt distribution throughout a cured ham could be obtained.

The technique of the method proposed by the authors differs from that of the other sampling methods discussed in that the muscle segments that appear in a cross-section slice of the ham are used as the source of sample material. This material, since it is related directly to the anatomy of the ham, eliminates the use of arbitrary measurements for sampling that are based on such varying characteristics as the size and shape of the ham.

Evidence of a difference in the chemical composition of muscles of the same ham is presented, and the possible significance of this in relation to salt absorption is pointed out.

The improved sampling method was applied to several hams during the curing process. Cross-section slices taken between the aitchbone and stifle joint were obtained from each ham. Within this area five uniform slices were obtained, and, beginning at the aitchbone, the first three were found to contain about 70 percent of the total meat examined. Of the 10 muscles sampled, 4—the semimembranosus, biceps femoris, semitendinosus, and rectus femoris—contributed 65 percent of the total meat. Since slice 2 represented the thickest and widest part of the ham, contained segments of all the muscles between the aitchbone and stifle joint, and possessed an average salt and water content, this slice was selected as the most suitable for sampling purposes.

Certain practical features of the analytical procedure are discussed. Advantages pointed out consist in the cutting up of the muscle segments with scissors prior to desiccation and in comminuting segments

from muscles after the removal of water, particularly in the case of muscles containing large quantities of fat, wherein difficulty in properly mixing lean and fat in the ordinary meat grinder is often experienced.

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CHANGES INDUCED IN THE BLOOD CELLS OF THE SOUTHERN ARMYWORM (PRODENIA ERIDANIA) BY THE ADMINISTRATION OF POISONS [1]

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INTRODUCTION

The results of a study of a histochemically detectable polysaccharide, considered to be glycogen, in the blood cells of unpoisoned southern armyworms (*Prodenia eridania* (Cram.) have been presented in a previous paper (*31*).[2] The present paper describes the effects upon the blood cells and blood-cell glycogen of administering poisons to the larvae of that insect. The poisons used were nicotine bentonite, nicotine peat, rotenone, pyrethrum, phenothiazine, barium fluosilicate, sodium fluoaluminate, sodium fluoride, mercuric chloride, calcium arsenate, calcium arsenite, arsenic trioxide, paris green, and lead arsenate. This investigation was qualitative and the results are reported as first approximations only.

MATERIALS

The nicotine bentonite and the nicotine peat each contained about 10 percent of nicotine. The rotenone was of a c.p. grade. The pyrethrum consisted of powdered pyrethrum flowers containing about 0.66 percent of pyrethrins I and II, including approximately 0.29 percent of pyrethrin I. The phenothiazine was approximately 99.5 percent pure. The barium fluosilicate was a fine-grade commercial product. The sodium fluoaluminate was synthetic and contained some uncombined aluminum oxide. The sodium fluoride conformed to the specifications of the American Chemical Society. The mercuric chloride was of a U. S. P. grade. The calcium arsenate was of average commercial grade, containing about 70 percent of arsenate and about 4 percent of arsenite. The calcium arsenite was about 85 percent pure. The arsenic trioxide was of a relatively pure grade. The paris green was a fine fraction separated from a commercial lot. The lead arsenate was a high-grade product. Administration of these poisons was by feeding. Nicotine was also administered as a vapor.

The insects used were sixth instars of the southern armyworm, reared in cages kept at approximately 25°–30° C. in winter and slightly higher in summer. The larvae were apparently in healthy condition.

METHODS

SAMPLING, SMEARING, FIXING, AND STAINING OF BLOOD

Sampling, smearing, fixing, and staining of blood were performed in the manner already described (*31*). To prevent the blood cells from undergoing form changes during sampling and smearing, larvae were heat-fixed by immersion in water at 60° C. for 5 minutes. The heat-fixed blood, obtained by piercing a proleg, was smeared on cover slips, air-dried, and stained by the Bauer technique, with thionin as a counterstain. The glycogen method of Best, the mucicarmine method of Mayer, and the method of salivary counterproof were not used in this investigation, but a number of duplicate blood preparations were stained for glycogen by the Lugol iodine method. The blood smears were made from larvae that remained alive until heat-fixed, whether or not they had received poison. Larvae that showed no body movement, no regurgitation, and no defecation upon being heat-fixed were considered to be dead and were not utilized.

NONLIGATURED LARVAE

In a few experiments larvae were fed turnip-leaf sandwiches containing cornstarch paste (previously boiled) and glucose, to build up blood-cell glycogen (*31*). Some of these larvae, used as controls, were fed turnip-leaf-cornstarch sandwiches without the glucose. Others were fed turnip-leaf-cornstarch sandwiches without the glucose but with a large amount of a poison mixed with the cornstarch paste. After the larvae had been allowed access to the unpoisoned and poisoned foods for various periods ranging approximately from 1 to 30 hours, blood smears were made and stained for glycogen by the Bauer method.

In other experiments larvae were subjected to nicotine vapor at room temperature by being placed upon a wire-mesh platform within a closed Petri dish, the bottom of which was covered with liquid nicotine. They did not touch the liquid. The air in the Petri dish was considered to be approximately saturated with the nicotine vapor. Control larvae were similarly placed in Petri dishes but without nicotine. The larvae subjected to nicotine behaved abnormally and, after an exposure of 23.5 or more hours, appeared shrunken and somewhat dehydrated. The controls, however, continued to appear normal. Several other larvae, placed for 24 hours in a desiccator over calcium chloride, appeared dehydrated but not so abnormal as those subjected for 23.5 hours to nicotine vapor. Blood smears from all these larvae were stained for glycogen.

LIGATURED LARVAE

In experiments involving ligatured larvae, the insects were first given access, usually for 16.5 hours (overnight), to turnip-leaf-cornstarch-glucose sandwiches to build up blood-cell glycogen, and immediately thereafter they were starved for about 2 hours. The larvae were then ligatured, and some were used as ligatured controls and others as ligatured poisoned larvae. The latter will be referred to as poisoned larvae whether or not poison administration was followed by hematological changes.

The ligature consisted of a string tied tightly about the larva's body, separating it approximately into an anterior (fore) two-thirds
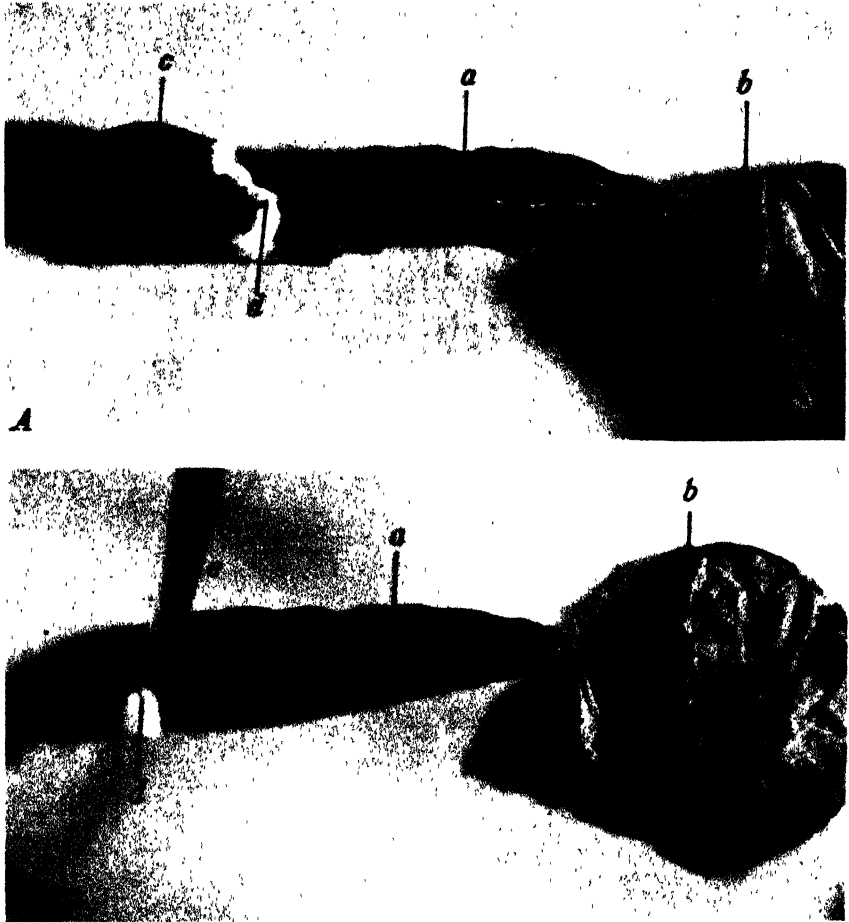
Ligatured larvae of *Prodenia eridania*. The fore end of each ligatured body is shown at *a*, near a turnip-leaf sandwich, *b*; the ligature is at *d*. In *A* the hind end is at *c*; in *B* it is on the other side of the cardboard stock, *e*, which encircles the body at the ligature.

to three-fourths and a posterior (hind) one-third to one-fourth portion Ligaturing caused regurgitation, defecation, and loss of coordination between the anterior and posterior parts of the body. Two ligatured larvae are shown in plate 1.

The ligatured control larvae were given access to unpoisoned turnip-leaf-cornstarch sandwiches for periods ranging from 4.5 to 28 hours, after which they were heat-fixed. The ligatured larvae to be poisoned were given access to turnip-leaf sandwiches containing cornstarch and a large quantity of poison for periods usually ranging from 3 to 28 hours, although the time was longer for several larvae. Blood smears from the fore and hind parts of the ligatured bodies of both the control and the poisoned larvae were then stained for glycogen.

The ligatured larvae ate unpoisoned food moderately well, but it was more difficult to get them to eat sandwiches containing lead arsenate, sodium fluoride, arsenic trioxide, mercuric chloride, and pyrethrum. When the sandwiches contained nicotine peat, nicotine bentonite, or, particularly, rotenone, the larvae seemed to eat about as readily as the control larvae ate unpoisoned sandwiches. The sandwiches that contained the remaining poisons were eaten less readily.

Because of the tendency of the larvae not to eat mercuric chloride and pyrethrum, these poisons, moistened with water, were applied with a brush to the mouth parts and the anterior legs of some of the larvae, which were thus made to ingest some of the material. Some of the larvae given these two poisons were held in cardboard stocks (pl. 1, *B*), which acted as shields preventing the poison from coming in contact with the hind parts of the ligatured bodies.

GLYCOGEN COUNTS AND INDICES

Glycogen counts and glycogen indices were determined as described in a previous paper (*31*). A glycogen index of 0 means that less than 1 out of 400 blood cells contained glycogen inclusions that were visible with the optical system used.

This investigation was begun without knowledge as to whether adequate glycogen counts and glycogen indices could be obtained. The experiments were performed with no particular intent of subjecting the results to statistical analysis. Nevertheless, the groups of glycogen indices from the ligatured larvae were eventually treated as follows: The mean glycogen index of the hind ends (M_H), the mean of the fore ends (M_F), the mean difference between these indices (M_{H-F}), and the standard deviation of these differences (SD_{H-F}) were calculated for the group of ligatured control larvae and for each group of ligatured poisoned larvae. The differences (*D*) in the value M_{H-F} between each group of ligatured poisoned larvae and the group of ligatured control larvae were obtained. The standard errors of these differences (SE_D) were calculated. The statistical evidence of the effects or lack of effects of the poisons on blood-cell glycogen thus obtained is regarded as only supplemental to the results of the cytological observations.

RESULTS

The results of the experiments consist of observations of hematological and cytological changes that followed poison administration,

and of changes in glycogen counts and glycogen indices. The descriptions of microscopic changes represent what is considered to be a logical arrangement of the observed hematological facts.

GENERAL HEMATOLOGICAL CHANGES

The blood cells of larvae treated with nicotine bentonite, nicotine peat, rotenone, phenothiazine, and pyrethrum appeared to be unaffected by these poisons, as indicated by comparison of the blood smears from the fore ends with those from the hind ends of the ligatured poisoned larvae and with those from the ligatured controls and the unligatured controls, although some blood cells showed a slight tendency toward passive-active changes, particularly when pyrethrum was used. The blood cells of larvae kept in concentrated nicotine vapor for 6 hours or less showed no effect of this treatment (pl. 2, *A*), but after 23.5 hours or more the blood cells of some of the larvae appeared abnormal relative to their controls, particularly with respect to rounding, agglutination, and increased vacuolization (pl. 2, *B*).

Administration of calcium arsenate, calcium arsenite, arsenic trioxide, paris green, lead arsenate, barium fluosilicate, sodium fluoaluminate, sodium fluoride, and mercuric chloride caused changes in the blood cells of the larvae receiving these poisons, which will be referred to in this paper as the effective poisons. Because individual larvae ingested different amounts of poisoned food, even when given access to it for the same period, no particular relationship between feeding period and hematological change was apparent. No attempt was made to determine the quantity of poison ingested or the relation between quantity ingested and hematological effect. The general hematological changes subsequent to poison administration, however, were recognized as progressive stages, which were designated as slight (+), moderate (++), and marked (+++). Plate 3, *A*, *B*, and *C*, and some of the other illustrations in this and in a previous paper (*31*) represent the normal (0) stage.

The slight, or +, stage of hematological change was characterized by a rounding up of most of the plasmatocytes and cystocytes, some cell agglutination, and perhaps an occasional spread form (pl. 4, *B*). The moderate, or ++, stage was characterized by the rounding up of the plasmatocytes and cystocytes, a considerable amount of cell agglutination, some cell distortion, and usually some cell disintegration (pl. 3, *D*). The marked, or +++, stage was characterized by the plasmatocytes and cystocytes having rounded or more or less spread forms, much cell agglutination, considerable cell distortion and disintegration, and frequently an obviously small number of cells in a smear (pl. 5, *B*, *C* and *E*).

Because the figures show only small areas of the blood smears from which they were taken, they do not identify the three stages so adequately as is possible by the more extensive microscopic examinations of the entire smears. Furthermore, the cytological details are not shown so clearly in the photomicrographs as they appeared in the smears. The figures, therefore, are presented only as illustrations, not as prototypes, of the three stages of general hematological change.

The terms employed in describing the three stages of general hematological change are used with the following meanings. Plasmatocytes and cystocytes are two types of southern armyworm blood cells that previously (*31*) have been identified among those that tend most

PLATE 2

Change in Blood Cells of the Southern Armyworm
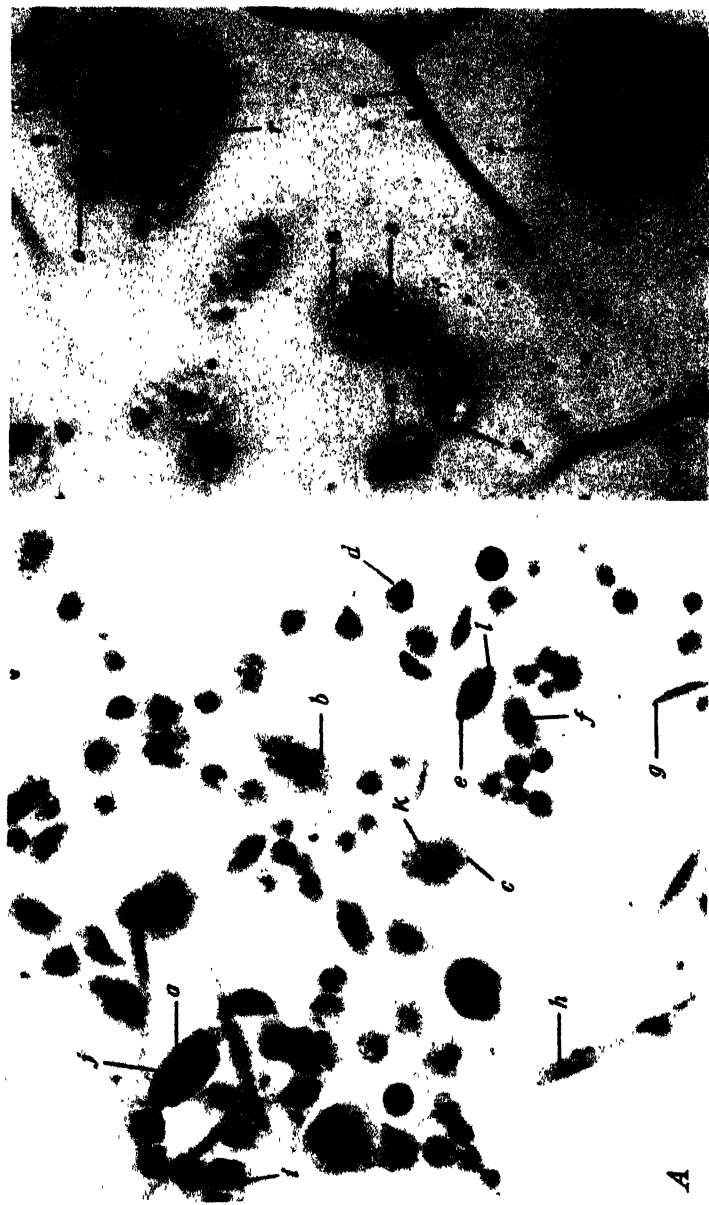
Parts of blood smears from unligatured larvae exposed to nicotine vapor: *A*, 6-hour exposure; *B*, 23.5-hour exposure. *a, b, c,* Cystocytes; *d, e, f,* plasmatocytes; *g, h,* nematocytes; *i–l,* glycogen inclusions; *m, n, o,* abnormal vacuolization; *p, q,* eccentric nuclei; *r, s, t,* abnormal agglutination; *u,* fold in plasma.
  Glycogen indices: *A*, 38.00; *B*, 5.00 percent.
  Bauer technique; 93× objective (oil); 10× ocular.
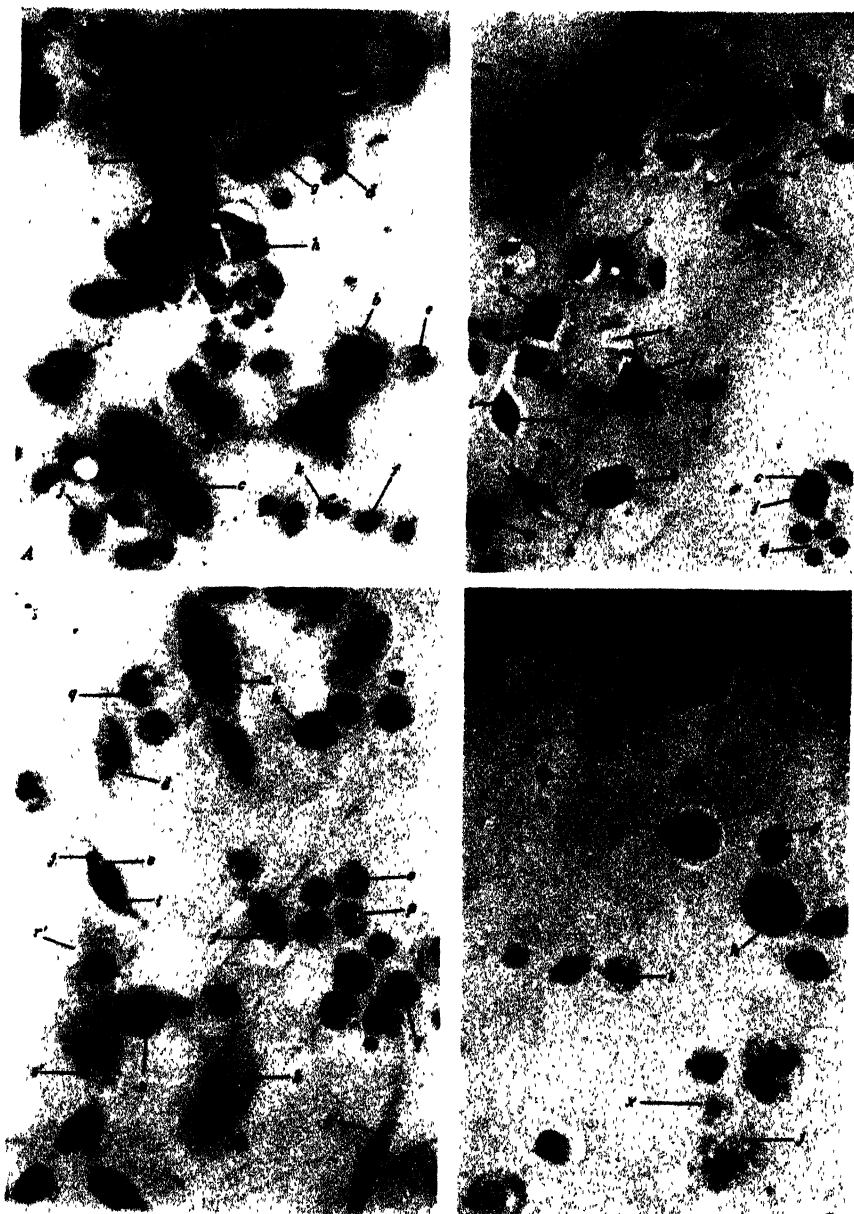
Parts of blood smears from a ligatured unpoisoned larva (*A*, hind end; *B*, fore end) and from a ligatured larva poisoned with mercuric chloride (*C*, hind end; *D*, fore end). *a, b, c*, Cystocytes; *d, e, f*, plasmatocytes; *g–k*, glycogen inclusions; *l, m*, polypodocytes; *n*, oenocytoid; *o, p, q*, spheroidocytes; *r, s, t*, breaks in thick smear of plasma; *u*, degenerating cell; *v*, rounded cell; *w*, eccentric nucleus; *x*, achromophilic cell; *y*, rounded, spread cells showing ectoplasm at *z; k'*, nematocytelike cell; *r'* and *s'*, cells with punctate nucleus.

   Stage of general hematological change: *A, B,* and *C*, 0; *D*, + +.
   Glycogen indices: *A*, 60.75; *B*, 86.75; *C*, 42.50; *D*, 18.25 percent.
   Bauer technique; 93× objective (oil); ocular 10× (*A* and *B*), 15× (*C* and *D*).
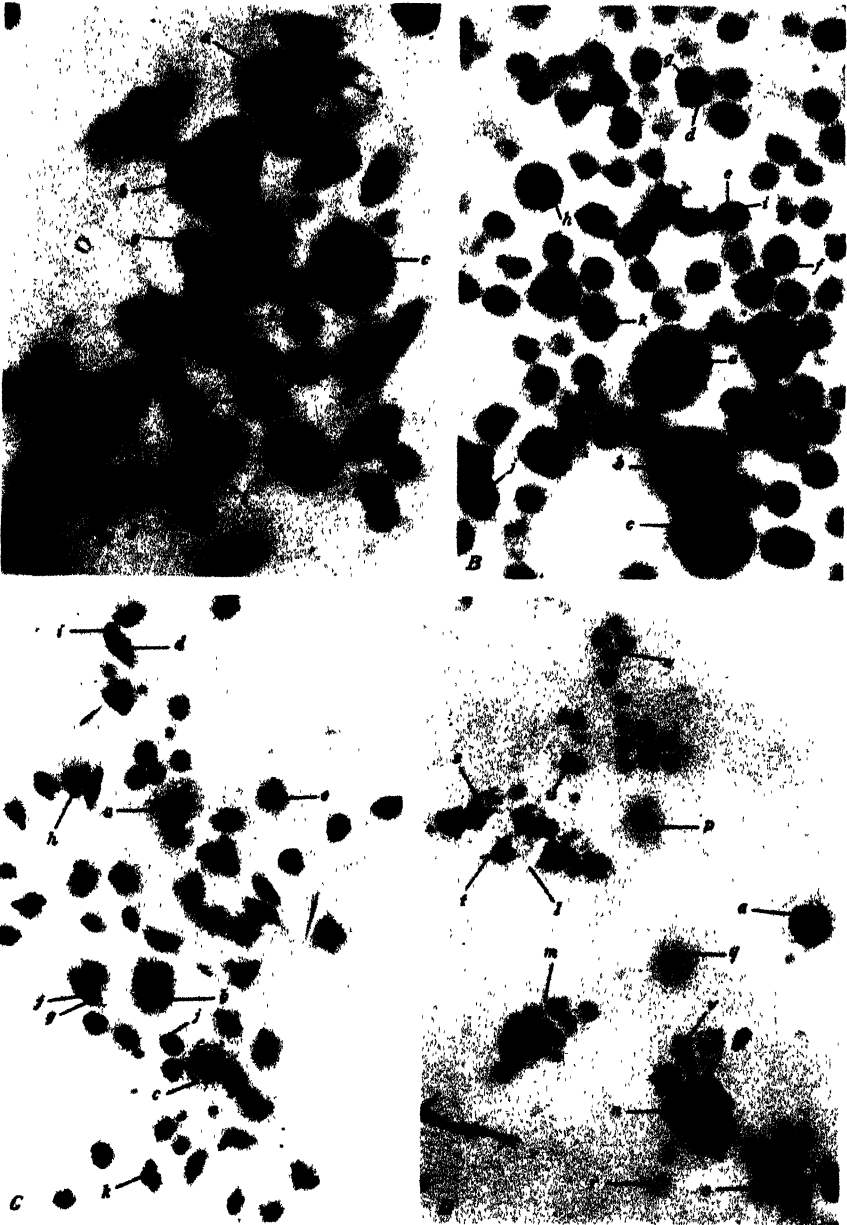
Parts of blood smears from two ligatured larvae poisoned with paris green: *A* and
B, hind and fore ends of one larva; *C* and *D*, hind and fore ends of the other larva.
a, b, c, Cystocytes; *d, e, f,* plasmatocytes; *g–k,* glycogen inclusions; *l–o,* abnormal agglutination; *p–r,* achromophilic cells; *s–v,* cellular deformation and plastid
formation; *w,* undeformed but degenerating spheroidocytes.
  Stage of general hematological change: *A* and *C,* 0; *B,* +; *D,* +++.
  Glycogen indices: *A,* 38.75; *B,* 31.00; *C,* 67.25; *D,* 2.00 percent.
  Bauer technique; 93× objective (oil); ocular 15× (*A* and *B*), 10× (*C* and *D*).
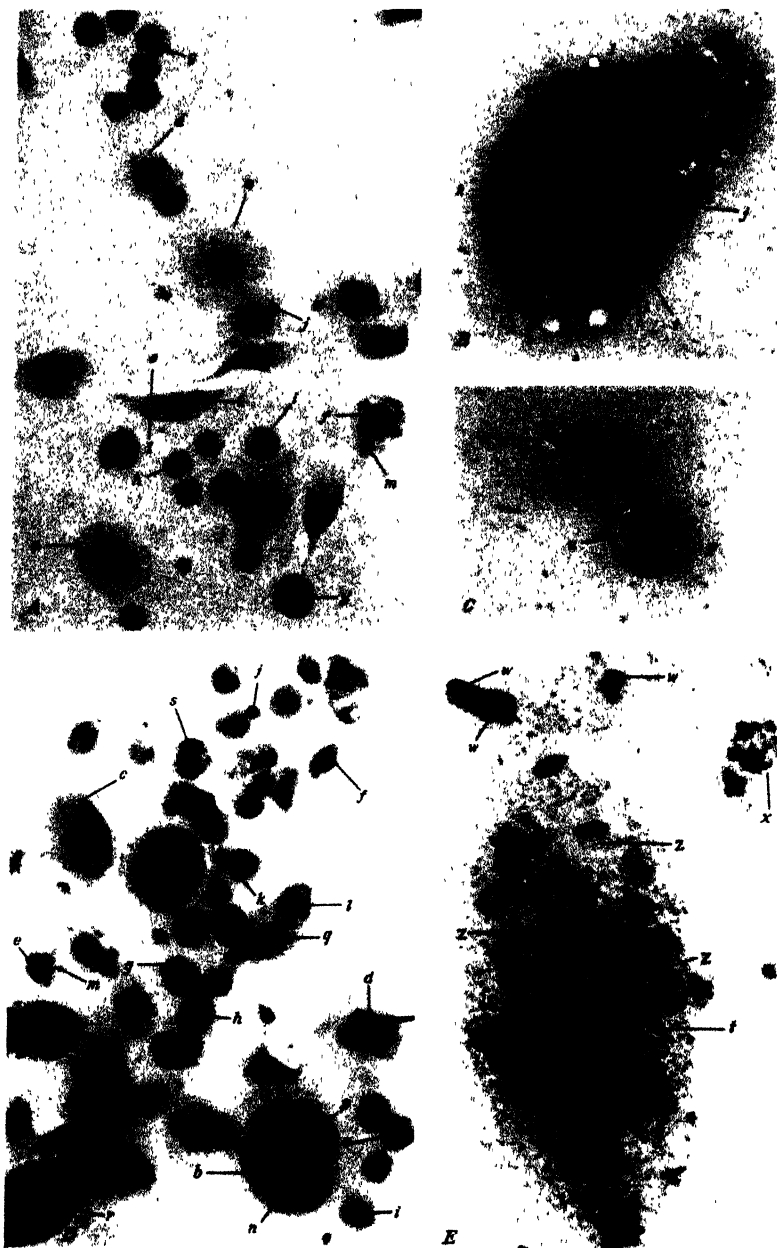
Parts of blood smears from two ligatured larvae poisoned with barium fluosilicate
(*A*, hind end; *B* and *C*, fore end) and calcium arsenite (*D*, hind end; *E*, fore end).
a, *b*, *c*, Cystocytes; *d*, *e*, *f*, plasmatocytes; *g*, *h*, *i*, spheroidocytes; *j–o*, glycogen
inclusions; *p*, *q*, *r*, apparent prophases; *s*, anaphase; *t*, degenerating agglutinated
cells; *u*, degenerating cystocyte; *v*, nearly completely degenerated cell; *w*,
degenerating cells; *x*, degenerating cell showing plastid formation; *y*, meta-
phase; *z*, somewhat swollen translucent cells, probably cystocytes.
    Stage of general hematological change: *A*, 0; *B*, *C*, and *E*, + + +; *D*, 0 (?).
    Glycogen indices: *A*, 34.00; *B* and *C*, 7.00; *D*, 43.75; *E*, 0.75 percent.
    Bauer technique; 93× objective (oil); 15× ocular.
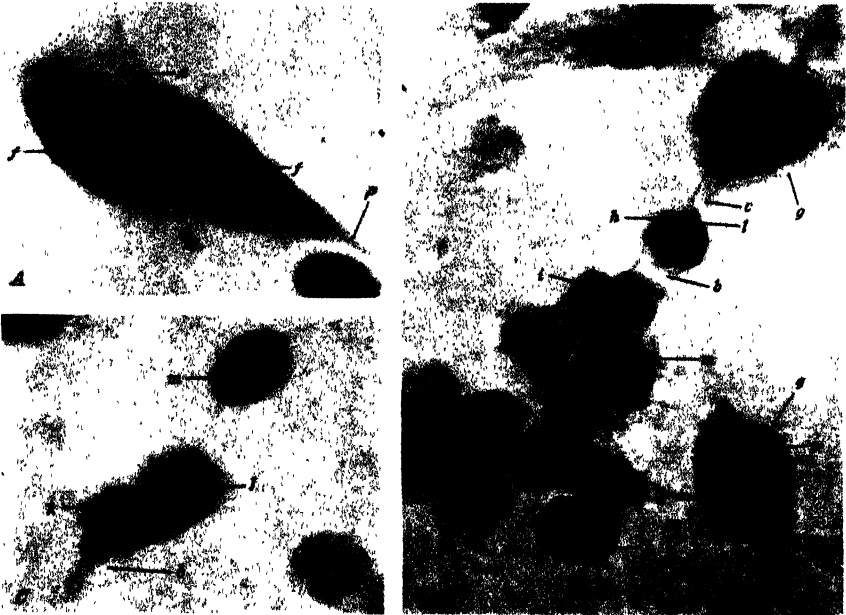
Parts of blood smears from ligatured larvae poisoned with mercuric chloride, showing cytoplasmic spreading: *A*, From the hind end, two plasmatocytes that appear to be in process of spreading (*a*) without losing their passive aspect or their general cytoplasmic structure and form; *B*, from the fore end, blood cells showing formation of thin ectoplasmic lamellae (*b–e*); *C*, from the fore end, three rounded plasmatocytes exhibiting ectoplasmatic activity (*b*). *f*, Nuclei; *g*, cystocytes; *h–j*, plasmatocytes; *k o*, glycogen inclusions; *p*, spindle end of cell.

   Stage of general hematological change: *B* and *C*, †.
   Glycogen index: *C*, 28.25 percent.
   Bauer technique; 93 × objective (oil); 15 × ocular.
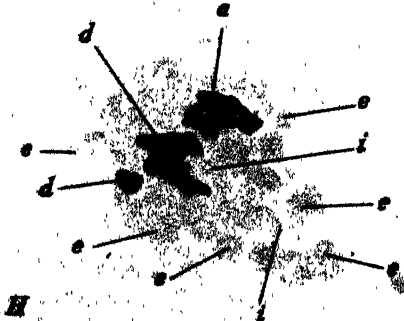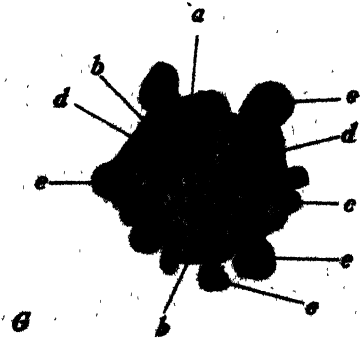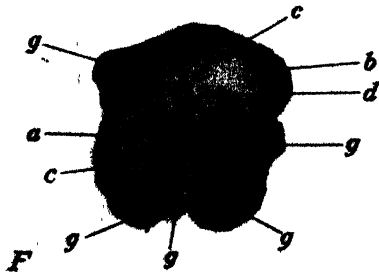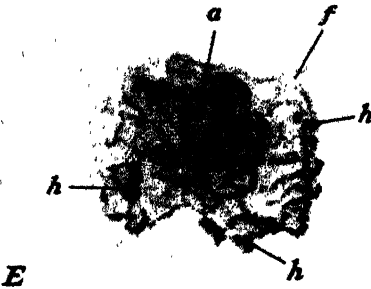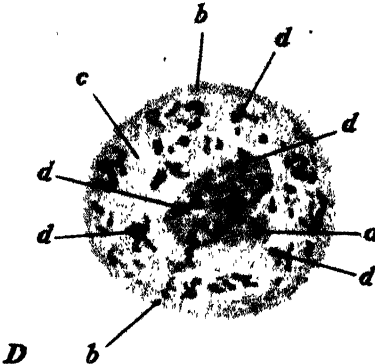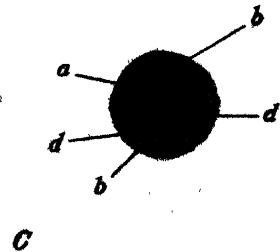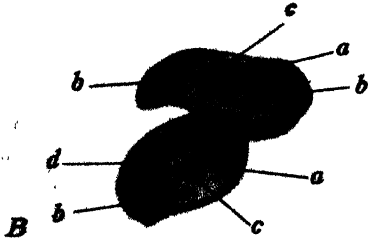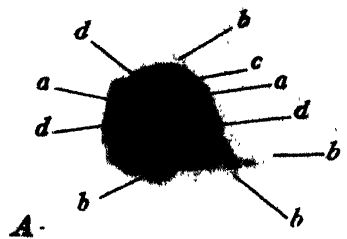
PLATE 7

Blood cells from the fore ends of ligatured larvae poisoned by barium fluosilicate (*A, B*), paris green (*C, D, F, G, H*), and mercuric chloride (*E*). *a,* Nucleus or part of nucleus; *b,* ectoplasm; *c,* endoplasm; *d,* glycogen inclusions; *e,* plastids; *f,* ragged ectoplasm; *g,* pseudopodialike bulges; *h,* granulation or minute plastids; *i,* remnant of main body of cytoplasm.

A, A rounded plasmatocyte showing irregularity of surface and extensions of ectoplasm, deeply stained endoplasm containing three glycogen inclusions, and the chromatin masses of the obscured nucleus.

B, Two plasmatocytes showing a pronounced demarcation between the homogeneous, relatively chromophilic ectoplasm and the endoplasm; widely spread chromatin masses of the nucleus are faintly visible in the endoplasm of each cell.

C, A rounded plasmatocyte, relatively chromophilic and containing two glycogen inclusions; cytoplasm partly obscuring nucleus and not particularly vacuolated but showing irregular intensity of stain; ectoplasm faintly distinguishable.

D, A cystocyte having a round, swollen, smooth-surface aspect, a partly obscured nucleus, many irregular glycogen inclusions, and an evenly distributed layer of ectoplasm.

E, A degenerating plasmatocyte showing cytoplasmic granulation (formation of minute plastids), cytoplasmic raggedness, and a somewhat distorted and relatively amorphous nucleus.

F, A cystocyte showing the formation of broad pseudopodialike bulges, a somewhat eccentric nucleus that shows a tendency toward pycnosis, and a faint remnant of a glycogen inclusion in the endoplasm, which is nearly structureless.

G, A rounded cystocyte showing plastid formation, a grossly punctate nucleus, and two remnants of glycogen inclusions in the endoplasm, which shows traces of cytoplasmic structures and a diffuse glycogen (Bauer) stain.

H, A degenerating plasmatocyte, with achromophilic cytoplasm, showing marked plastic formation, a somewhat ragged nucleus, and a remnant of the main body of the cytoplasm containing two glycogen masses.

Stage of general hematological change: *A, B,* and *C,* +; *D* and *H,* ++; *E, F,* and *G,* +++.

Glycogen indices: *A* and *B,* 25.50 (fore end) and 24.75 (hind end); *C,* 31.00 (fore end) and 38.75 (hind end); *D, F, G,* and *H,* 0 (fore end) and 21.75 (hind end); *E,* 12.75 (fore end) and 40.75 (hind end) percent.

Bauer-thionin technique; oil immersion; camera lucida wash drawings.

Blood cells from the fore ends of ligatured larvae poisoned with mercuric chloride (*A, C, D, F J*) and paris green (*B, E*). *a,* Nucleus; *b,* plastid formation; *c,* gross vacuoles; *d,* glycogen inclusions; *e,* pseudopodialike bulges of cytoplasm; *f,* ectoplasm; *g,* endoplasm; *h,* nuclear extrusion; *i,* thionin-stained granules; *j,* vacuolization of glycogen inclusions; *k,* nuclear polysaccharide; *l,* a large central area (endoplasm?).

A, A large, degenerating cell of questionable identification showing marked plastid formation, faint indications of gross vacuolation of the cytoplasm and of some of the plastids, and a nucleus with an aspect suggestive of mitosis

B, A degenerating, achromophilic plasmatocyte showing marked plastid formation.

C, A degenerating, rounded plasmatocyte with marked cytoplasmic vacuolization.

D, A degenerating, rounded plasmatocyte with an eccentrically located pycnotic nucleus, cytoplasmic pseudopodialike bulges, and glycogen inclusions

E, A degenerating cystocyte showing partial extrusion of a somewhat pycnotic nucleus.

F, A large round, swollen cystocyte showing nuclear extrusion

G, Either a large plastid or an enucleated plasmatocyte that contains a single glycogen inclusion.

H, A rounded plasmatocyte showing nuclear fragmentation.

I, A round, swollen blood cell of problematical identification, although probably a plasmatocyte, containing glycogen inclusions

J, A cell of problematical identification with nuclear polysaccharide, probably glycogen, cytoplasmic achromophilia, loss of structural organization, and gross, irregular vacuolization.

Stage of general hematological change: *A, B, C, E, F, H, I, J,* + + + : *D, G,* + +.

Glycogen indices: *A, C, F, H, I, J,* 12.75 (fore end) and 40.75 (hind end), *B* and *E,* 0 (fore end) and 21.75 (hind end); *D* and *G,* 18.25 (fore end) and 42.50 (hind end) percent

Bauer-thionin technique; oil immersion; camera lucida wash drawings.
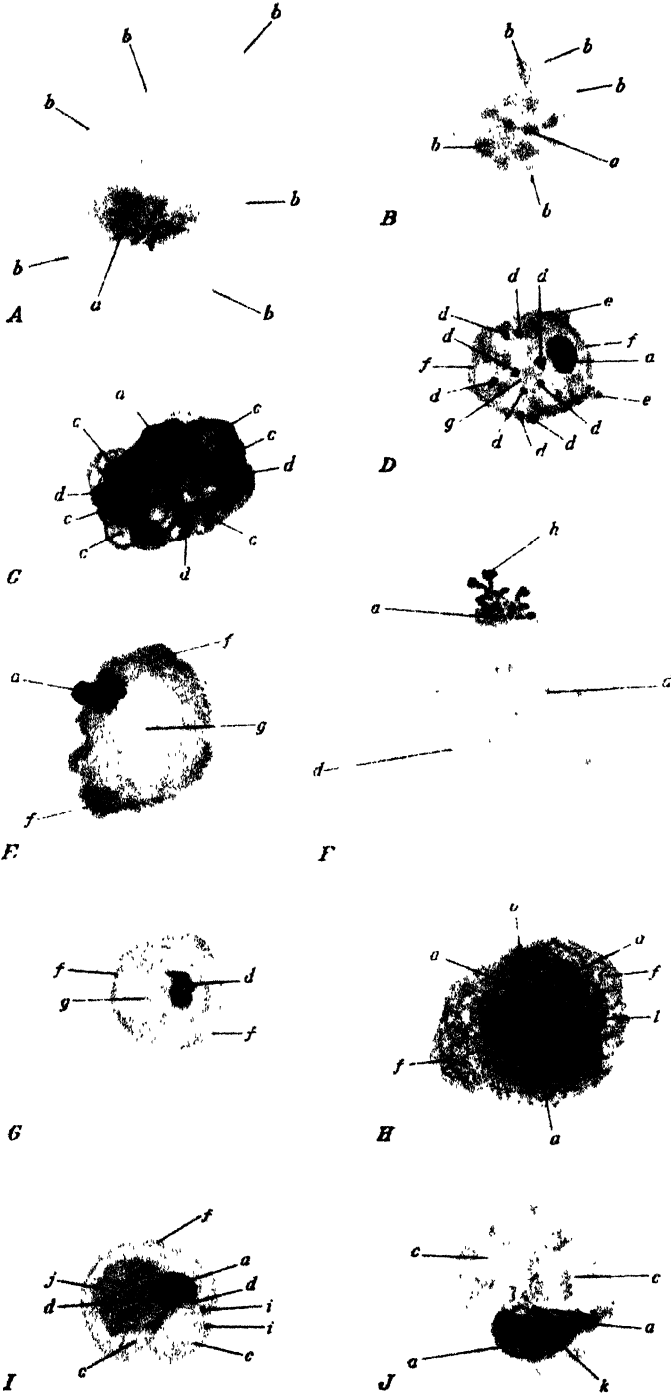
readily to contain glycogen inclusions (pls. 2, *A;* 3, *A, B,* and *C;* 4, *A, B,* and *C;* 5, *A* and *D;* 6). Rounding up is illustrated by the withdrawal of the spindle ends of a fusiform cell, the contour of which usually tends to become but may not actually become circular (pl. 4, *A* and *B*). Cell agglutination, or coagulation, applies to the aggregation of certain cells into clumps, such as occurs during the clotting of insect blood (*30, 32;* pls. 4, *D;* 5, *B* and *E;* 6). Distortion designates the occurrence of cellular forms that are apparently not characteristic of normal blood cells in either their passive or their active state (pls. 7, *F;* 8, *E*). Disintegration refers to the more advanced stages of degeneration, in which the cell may show a tendency to lose part of its protoplasm or may become more or less ragged, broken down, or structureless (pls. 4, *D;* 7, *E, G,* and *H;* 8, *A, B, I,* and *J*). Spread form is that form characteristically associated with the active state of certain blood cells, in which they tend to become thinner and to occupy greater areas of the surfaces on which they rest, and in which their homogeneous, peripheral cytoplasm, or ectoplasm, frequently becomes quite distinguishable from their heterogeneous endoplasm, which contains nucleus, vacuoles, glycogen inclusions, and other formed or included bodies (pls. 3, *D, y;* 6, *B* and *C;* 7, *B*).

Each of the smears that showed the effects of poison administration presented blood pictures that were in accord with the progressive stages of general hematological change, whether the smear was from a ligatured or a nonligatured larva. It should be emphasized, however, that the stages +, + +, and + + + have been described in very general terms. Apparently not all the blood cells of poisoned larvae, for example, underwent passive-active transformations in which they first rounded up and later spread.

<center>CYTOLOGICAL CHANGES</center>

The cytological changes that followed the administration of the effective poisons fall into three categories: (1) Passive-active transformation and cell agglutination, (2) possible regenerative changes, and (3) degenerative changes. Unless otherwise stated, this description is limited to the cystocytes and the plasmatocytes, because these cell types, particularly the cystocytes, could be readily identified in the Bauer-stained thionin-counterstained smears. As previously indicated (*31*), the cystocytes and the plasmatocytes are essentially fusiform cells, although some of them may assume roundish, ovoid, or polymorphic forms in the circulating blood.

<center>PASSIVE-ACTIVE TRANSFORMATION AND CELL AGGLUTINATION</center>

Beginning with a spindle-shaped cystocyte or plasmatocyte, the changes described below were found to follow the administration of a sufficient quantity of an effective poison. The fusiform cells (passive form) tended to undergo transformation, which left them partly or completely in what were considered to be their active forms. During passive-active transformation a fusiform cell (pls. 3, *C, a, b,* and *e;* 4, *C;* 5, *A, e*) may withdraw its spindle ends and round up, assuming a more or less circular (pl. 4, *B*), ovoid (pl. 3, *D;* 7, *B*), or polymorphic (pls. 6, *B,* and *C;* 7, *A*) outline, or it may begin to spread without withdrawing its spindle ends (pl. 6, *A*). The transformation frequently involves augmented activity of the cytoplasm nearest the

cell surface, resulting in the development of surface irregularities and various extensions of the ectoplasm (pls. 6, *B* and *C; 7, A*).

After partial or complete transformation the cells presented variants of the following forms:

(1) Variably round or ovoid outline and exhibiting surface irregularities (pl. 7, *A*).

(2) More or less circular, smooth outline, without surface irregularities. The heterogeneous endoplasm could be readily distinguished from the surrounding homogeneous ectoplasm (pls. 3, *B*, *D*, *z; 7, B*), which varied from a very thin to a moderately thick layer, locally thinned or thickened into tonguelike or caplike enlargements, probably representing broad pseudopodial formations. In this form of cell the ectoplasm often tended to be more chromophilic than the endoplasm. Sometimes this cellular form presented a swollen or turgid aspect.

(3) Round or nearly round without marked distinction between endoplasm and ectoplasm and without particular surface irregularities (pls. 4, *B; 7, C*).

(4) More or less spread, having a round, ovoid, or irregular shape, and showing much evidence of surface activity, in the form of very thin, lamellar, ectoplasmic extensions or membranes of the same character as those shown in plate 6, *B* and *C*, some of which were relatively large.

(5) Tending to maintain a passive aspect, except that they appeared to have become much more spread upon the surfaces whereon they lay (pl. 6, *A*).

The cystocytes tended to assume forms (1), (2), and (3). The plasmatocytes tended to assume any one of the five forms.

Accompanying these changes and the subsequent degenerative changes, agglutination occurred involving various types of cells, among which were the cystocytes and the plasmatocytes (pls. 4, *D; 5, B* and *E; 6, B* and *C*).

POSSIBLE REGENERATIVE CHANGES

Changes of a regenerative character may have occurred rather soon after poison administration. The possible regenerative changes consisted of an apparent increase in mitotic activity, made evident in the nuclei of some cells by their assuming an aspect suggestive or definitely indicative of the prophase of mitosis (pls. 5, *D*, *p*, *q*, and *r*). Other phases of mitosis were also observed in blood smears from both normal and poison-affected larvae (pl. 5, *D*, *s*).

DEGENERATIVE CHANGES

The cytoplasmic changes of a degenerative character include apparent swelling, disruption of and decrease in visibility of normal structure, achromophilia, decrease and loss of blood-cell glycogen, formation of broad pseudopodia or cytoplasmic bulges, plastid formation, excessive vacuolization, and raggedness. Nuclear degeneration involved distortion, raggedness, loss of normal structure, achromophilia, assumption of more or less peripheral positions, fragmentation, pycnosis, and extrusion.

The plasmatocytes and the cystocytes frequently exhibited what appeared to be a swollen aspect, which seemed particularly associated

with a rounded, smooth-surface, cellular form and occurred more conspicuously among the cystocytes (pls. 5, *E*, 7, *D;* 8, *F* and *I*). Whether an actual increase in cell volume or a relatively great spreading of the cell upon the supporting surface was involved was not determined. Various degrees of apparent swelling occurred.

Disruption of and decrease in visibility of cytoplasmic structures were observed among both cystocytes and plasmatocytes. In general, as the active degenerative changes progressed, the normal cytoplasmic structure tended either to become disrupted and to assume an abnormal appearance or to become less visible until it completely disappeared; sometimes both changes occurred (pls. 5, *E*, *e*, *f*, *g*, and *w;* 7, *D*, *F*, *G*, and *H;* 8). With complete disappearance of normal endoplasmic structure the cytoplasm tended to assume a more or less homogeneous aspect, in which case the peripheral ectoplasm might or might not be distinguishable from the endoplasm. Usually as degeneration proceeded many of the affected cells became relatively achromophilic.

During cytoplasmic degeneration a decrease of blood-cell glycogen occurred in many cells, and with sufficient cellular degeneration it eventually disappeared (pls. 7, *F* and *G;* 8). The disappearance of the glycogen inclusions sometimes seemed to involve an appearance in the endoplasm of a faint, diffuse positive reaction to the Bauer technique, indicating the possibility of glycogen occurring there in a diffuse state. In some of the cells some glycogen persisted into rather advanced stages of degeneration, as shown in plate 7, *H*.

Some degenerating cells underwent a sort of deformation in which broad bulges, suggestive of pseudopodia, occurred (pls. 7, *F;* 8, *D* and *E*). Sometimes only such broad cytoplasmic bulges occurred, but sometimes cells formed a greater number of smaller bulges or pseudopodia (pls. 4, *D*, *s*, *t*, and *r;* 5, *E*, *r;* 7, *G*), which gave the peripheries of the cells a very irregular aspect, as though the cells were extending tongues of cytoplasm from their surfaces. At more advanced stages of this process some of the protruded cytoplasmic tongues occasionally became nearly or quite detached from the rest of the cell and lay near the cell surface as free bodies, or plastids (pls. 7, *H;* 8, *A* and *B*). Sometimes in the final stages of plastid formation by a disintegrating cell, most of the cytoplasm appeared as a number of more or less separated plastids. Plastids frequently had a relatively homogeneous appearance.

Cells frequently took on a more ragged aspect as degeneration proceeded, the cytoplasm appearing irregularly frayed and torn.

Degenerating cells were sometimes grossly vacuolated to an abnormal degree (pl. 8, *C*).

Resting nuclei of the plasmatocytes and cystocytes normally possess a decidedly punctate structure (pls. 3, *C;* 5, *A* and *D;* 6, *A;* and some of the illustrations in *31*). During degeneration many nuclei tended to lose their punctate structure and became distorted, ragged, amorphous, and achromophilic (pls. 7, *D*, *E*, and *F;* 8, *A*, *B*, *F*, and *J*). Some nuclei became more or less pycnotic (pl. 7, *F* and *G*), variably assumed peripheral positions (pls. 7, *G;* 8, *D* and *I*), and in some cells became partially or completely extruded (pl. 8, *E*). Sometimes nonpycnotic nuclei tended to become extruded (pl. 8, *F*). Occasionally nuclei apparently being extruded seemed to be in a

mitotic condition (pl. 8, *F*). Large masses of cytoplasm, with or without glycogen inclusions but containing no visible nuclei, were occasionally observed. Some of these masses were too large to be considered as mere plastids and were thought to be cells that had lost their nuclei, either by extrusion or by degeneration. Plate 8, *G*, shows such a nonnucleated cell, or perhaps plastid, that contains a single glycogen inclusion.

Nuclei of degenerating cells sometimes appeared to break up into a number of discrete bodies. The fragmented nuclei showed varying tendencies to scatter (pls. 8, *H;* 9, *A*). Occasionally either whole nuclei (pl. 8, *I*) or nuclear fragments appeared as homogeneous droplets.

OTHER OBSERVATIONS

In some degenerating cells the glycogen inclusions looked as though they might have become aggregated or fused into somewhat larger masses. Glycogen inclusions sometimes appeared especially vacuolated (see also *31*), particularly when mercuric chloride was administered. Not all the cystocytes and plasmatocytes in a blood smear showed plastid formation, even when plastid formation was most pronounced. Although many of the pycnotic nuclei occupied eccentric positions in the cells, complete nuclear extrusion was observed infrequently. Blood smears from some of the poisoned larvae contained very large cells, identified as variably abnormal midgut epithelial cells that were often excessively vacuolated, bacteria that apparently had entered the blood stream from the ruptured gut (pls. 3, *D;* 5, *B, C,* and *E*), and other structures that were interpreted as partly digested fragments of leaf. Such tissue and intestinal debris were observed particularly in the blood of larvae poisoned with lead arsenate, arsenic trioxide, and the fluorides. In some of the blood smears from poisoned larvae, particularly from those given lead arsenate, dark thionin-stained spots or bodies occurred either in the plasma or associated with some of the cells. Infrequently a cell appeared to be in the process of extruding glycogen inclusions.

In some of the blood smears showing advanced cellular degeneration, from larvae poisoned with mercuric chloride, the nuclei of certain cystocytelike cells of questionable identification showed a positive polysaccharide reaction to the Bauer stain (pls. 8, *J;* 9, *B*). These cells seemed to be either modified cystocytes or transformed plasmatocytes. Because certain transitional forms seemed to link these questionable cells with the plasmatocytes, at least some of them were tentatively considered to be modified plasmatocytes.

Although plastid formation was observed to occur in larvae poisoned with the arsenicals and with mercuric chloride, it was not definitely observed in larvae poisoned with the fluorides, although some blood cells showed formation of broad pseudopodia. The most marked plastid formation was observed after administration of paris green. Abnormal vacuolization of blood cells was observed particularly after administration of mercuric chloride and the fluorides.

In contrast to the cystocytes and plasmatocytes, the spheroidocytes (pls. 3, *B, o, p,* and *q; C, o, p,* and *q;* 5, *A, g, h,* and *i; D, g, h,* and *i;* plates in *31*) underwent little or no change in form as degeneration proceeded. No glycogen was observed in any oenocytoid (pl. 3, *B, n:* plates in *31*), either normal or degenerate. Nuclear polysaccharide was detected only in the indeterminate cystocytelike cells that have

PLATE 9
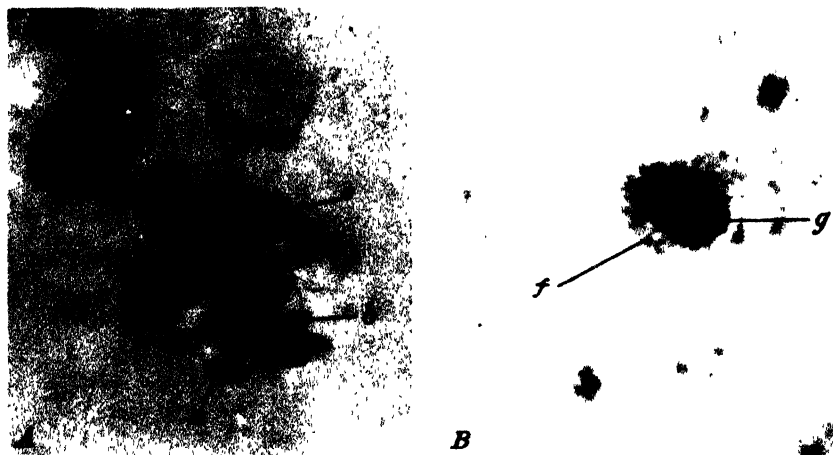
Blood cells from the fore ends of larvae poisoned with mercuric chloride: *A*, A cell showing nuclear fragmentation, as at *a*, *b*, and *c*; plastid formation is indicated at *d* and *e*. *B*, A cell showing in its nucleus (*f*) a positive reaction (*g*) to the Bauer stain for polysaccharide.

Both smears showed a + + + stage of general hematological change.

Bauer technique; 93 objective (oil); 15 × ocular.

been described, and then only when mercuric chloride was administered.

CHANGES IN GLYCOGEN INDICES AND GLYCOGEN COUNTS

GLYCOGEN INDICES

The statistical values for the glycogen indices of ligatured control and ligatured poisoned larvae are given in table 1.

TABLE 1.—*Difference between the mean glycogen indices of the hind ends and those of the fore ends of ligatured larvae of Prodenia eridania after the administration of unpoisoned (controls) and poisoned food, and differences between the poisoned larvae and the controls*

Treatment	Larvae	Mean glycogen index		Difference between hind and fore ends		Difference between poisoned and unpoisoned larvae	
		Hind end (M_H)	Fore end (M_F)	Mean difference (M_{H-F})	Standard deviation (SD_{H-F})	Difference from control (D)	Standard error (SE_D)
	Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
None (control)	21	32.33	35.58	−3.25	6.72		
Nicotine bentonite	11	53.18	54.66	−1.48	7.08	1.77	2.59
Nicotine peat	8	47.94	54.69	−6.75	17.34	3.50	6.30
Rotenone	8	18.69	24.19	−5.50	9.55	2.25	3.68
Pyrethrum	18	37.57	40.51	−2.94	11.27	.31	3.03
Phenothiazine	6	41.63	37.67	+3.96	6.05	7.21	2.87
Barium fluosilicate	13	32.90	24.32	+8.58	11.89	11.83	3.61
Sodium fluoaluminate	10	36.70	19.55	+17.15	15.22	20.40	5.03
Sodium fluoride	12	42.54	34.06	+8.48	10.18	11.73	3.28
Mercuric chloride	11	35.82	18.43	+17.39	20.96	20.64	6.49
Calcium arsenate	5	52.60	19.70	+32.90	5.07	36.15	2.69
Calcium arsenite	5	27.10	1.70	+25.40	14.23	28.65	6.53
Arsenic trioxide	15	49.62	34.95	+14.67	15.50	17.92	4.26
Paris green	9	37.66	17.91	+19.75	18.70	23.00	6.41
Lead arsenate	15	41.35	30.88	+10.47	16.76	13.72	4.57

It should be noted that the difference in the mean glycogen index between the hind ends and the fore ends (M_{H-F}) of the controls is a negative value, and that negative values of M_{H-F} were also obtained for nicotine bentonite, nicotine peat, rotenone, and pyrethrum, none of which, as has been stated, produced marked morphological changes in the blood cells. Although the M_{H-F} value for phenothiazine is positive, no definite hematological changes followed the administration of this poison. All the other poisons produced hematological changes, and all have positive M_{H-F} values.

Since the standard deviation of the hind-fore end differences (SD_{H-F}) of the controls is 6.72, the difference between the hind and the fore end ($H-F$) of a poisoned larva would have to be greater than +10.19 (i. e., 13.44−3.25) when 2 SD_{H-F} is taken or +16.91 (i. e., 20.16−3.25) when 3 SD_{H-F} is taken as a measure of significance, if the larva is to be regarded as significantly showing the result of poison administration by a relative decrease in fore-end glycogen index. The standard of 3 SD_{H-F} is used here. Only three poisoned larvae which had hind-end glycogen indices of 20 percent or more and whose hind-end and fore-end blood smears showed, respectively, 0 and ++ or 0 and +++ general hematological conditions showed differences between hind and fore ends of less than 17 percent. It was considered that in these experiments the $H-F$ difference in glycogen index for a poisoned larva must be at least +17 percent before the glycogen index of the fore end would show a significant relative decrease as a result

of poison administration. Inasmuch as this value is derived from the controls only, it should be considered as a minimum difference.

Three times SE_D is also taken as a standard of significance. Accordingly, administration of nicotine bentonite, nicotine peat, rotenone, pyrethrum, and phenothiazine is found to have no effect upon $H - F$ differences in glycogen index, since in each group D is less than 3 SE_D, whereas relative decreases in the fore-end glycogen index are found to follow administration of barium fluosilicate, sodium fluoaluminate, sodium fluoride, mercuric chloride, calcium aresnate, calcium arsenite, arsenic trioxide, paris green, and lead arsenate.

Figure 1 shows the frequency distribution of the $H - F$ differences in
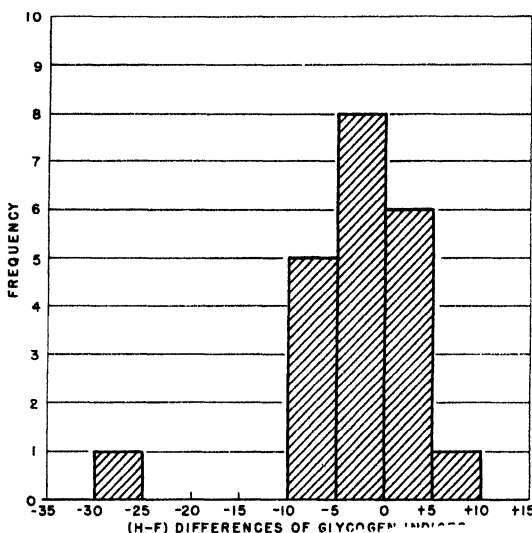
FIGURE 1.- Histogram showing frequency distribution of hind-fore end differences of 21 ligatured control larvae.

glycogen index for the group of 21 ligatured control larvae, when the class intervals are as shown in the figure. The -25 to -30 value was for a larva having H and F glycogen indices of 60.75 and 86.75 percent, respectively.

Most of the unligatured larvae constituted groups less suitable for statistical comparison. The mean glycogen index and the standard deviation for the 23 unligatured larvae subjected to nicotine vapor for various periods up to 29.5 hours were 42.61 and 14.24 percent, respectively; the corresponding values for the 5 unligatured controls were 50.00 and 11.81 percent. The difference between these mean glycogen indices is 7.39, and the standard error of this difference is 6.06. These values indicate that the larvae treated with nicotine vapor did not differ significantly from their controls, although some decrease in glycogen index might follow very prolonged treatment.

The values given in table 2 show the relationship between glycogen index and stage of general hematological change subsequent to administration of each of the effective poisons. Each hind-end value is an average glycogen index of the hind ends of all the ligatured larvae that yielded the corresponding fore-end values. The fore-end values are averages of the fore-end glycogen indices grouped according to

whether they showed 0, +, ++, or +++ general hematological change. These values indicate that the glycogen index tends to undergo a progressive decrease as the general hematological changes proceed, and that usually the most marked reductions of glycogen index occur during the ++ and +++ stages.

TABLE 2.—*Average glycogen indices corresponding to progressive stages of general hematological changes following poison administration to ligatured larvae of Prodenia eridania*

		Glycogen index of				
Poison		Hind end	Fore end with indicated stage of hematological change			
			0	+	++	+++
		Percent	*Percent*	*Percent*	*Percent*	*Percent*
Barium fluosilicate		32 90	34 00	19 50	16 56	7 00
Sodium fluoaluminate		36 70	39 00		14 25	7 16
Sodium fluoride		42 54	36 50	40 33	19 00	13 25
Mercuric chloride		35 82	32 31	28 25	18 25	11 70
Calcium arsenate		52 60		27 63	14 42	
Calcium arsenite		27 10				1 70
Arsenic trioxide		49 62	55 80	41 25	32 19	15 05
Paris green		37 66	35 38	30 63	9 42	1 00
Lead arsenate		41 35	57 88	50 58	15 25	6 85

GLYCOGEN COUNTS

The average glycogen counts of all the nonligatured larvae and of the hind ends plus fore ends of the ligatured control larvae are presented graphically in figure 2, *A*. It is shown that the curves from nonpoisoned larvae tend to have the form 0>1>2,>3<3+, where the numbers represent classes of the count and the symbols indicate their numerical relationship.[3] Figure 2, *B*, shows that the average
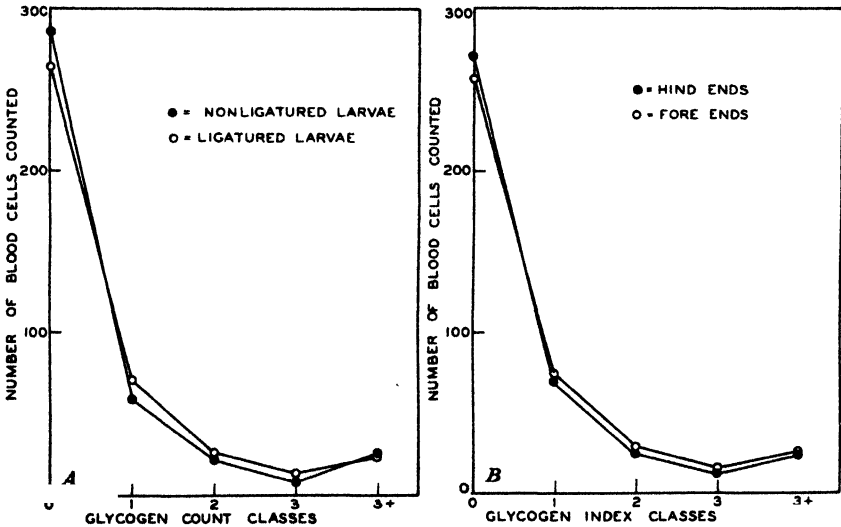
FIGURE 2.—Average glycogen counts from larvae fed turnip-leaf sandwiches containing carbohydrate but no poison (controls): *A*, Nonligatured and ligatured larvae; *B*, Hind and fore ends of ligatured larvae.

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[3] A discussion of the form of glycogen counts from unpoisoned larvae has been given in a previous paper (*31*).

glycogen counts of hind and fore ends are similar in form and that the counts from the fore ends may tend to exceed those from the hind ends in classes 1 to 3+, inclusive.

Average glycogen counts of the hind and fore ends of ligatured larvae given nicotine bentonite, nicotine peat, rotenone, and phenothiazine are shown in figure 3. All these counts have the usual $0>1>2>3<3+$ form. Unlike the corresponding curves of the controls (fig. 2, *B*), the hind-end and fore-end curves cross somewhere between classes 1 and 3+. Average glycogen counts of the hind ends, the fore ends that showed no definite general hematological change,
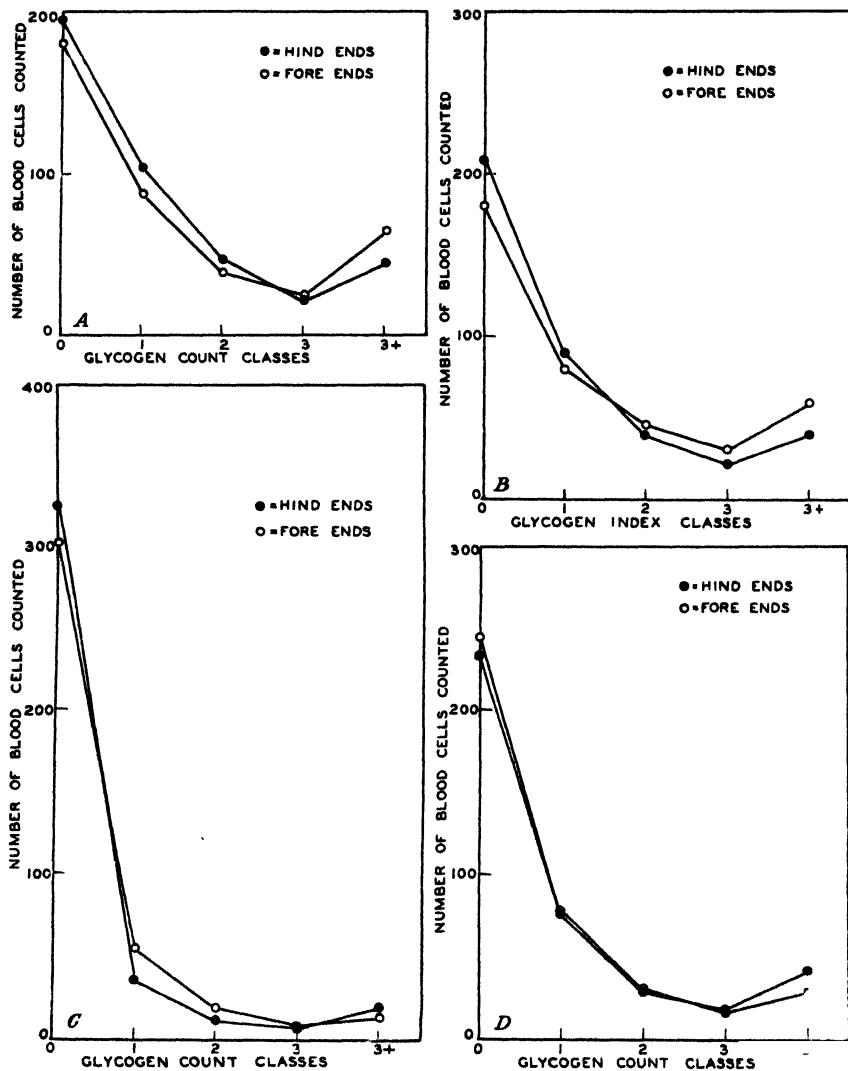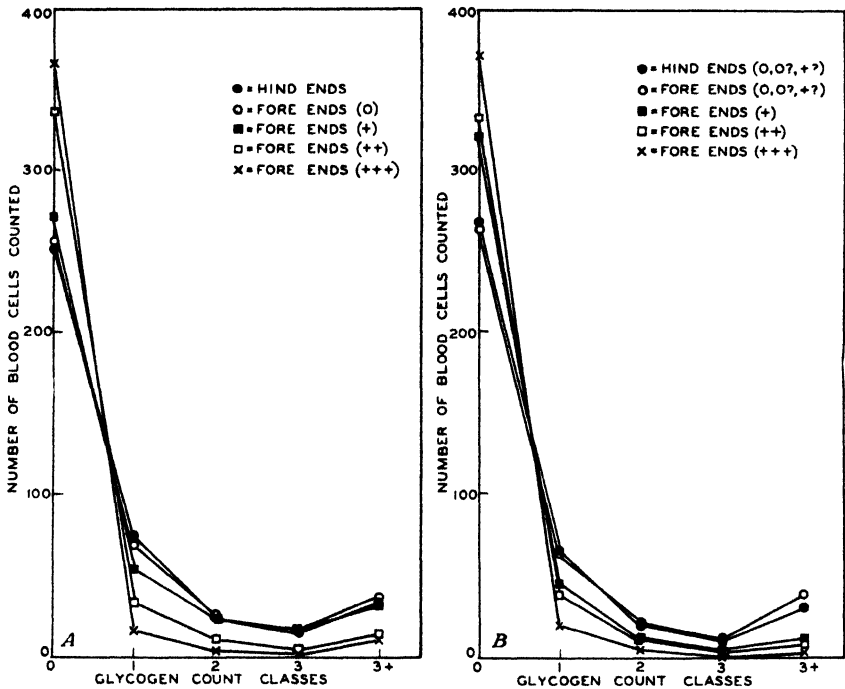

FIGURE 3.—Average glycogen counts from the hind and fore ends of larvae fed carbohydrate-turnip-leaf sandwiches, ligatured, and then fed cornstarch-turnip-leaf sandwiches containing the following poisons: *A*, Nicotine bentonite; *B*, nicotine peat; *C*, rotenone; *D*, phenothiazine.

and the fore ends that showed a + stage of general hematological change subsequent to pyrethrum administration are presented in figure 4. Crossing between classes 1 and 3+ also occurs in this figure.

A comparison of average glycogen counts (not illustrated) for the combined hind ends and for the combined fore ends of all the larvae given calcium arsenate, calcium arsenite, arsenic trioxide, paris green, and lead arsenate showed that the hind ends tended to exceed the fore ends in classes 1 through 3+. The corresponding average glycogen indices were 42.88 and 25.65 percent.

Figure 5 contains the average glycogen-count curves for the combined hind ends and for the fore ends grouped according to the type
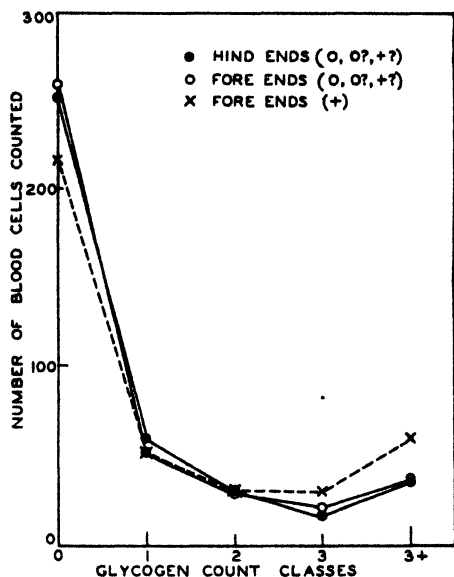
FIGURE 4 —-Average glycogen counts from hind and fore ends of larvae fed carbohydrate-turnip-leaf sandwiches, ligatured, and then given pyrethrum, or pyrethrum in cornstarch-turnip-leaf sandwiches. The fore ends are separated into those that showed no effect or a questionable + effect and those that definitely showed a + general hematological effect of poison administration.

of hematological change. The curves indicate that, relative to their hind ends, the 0 fore ends tended to have a somewhat larger number of glycogen inclusions, but that the +, ++, and +++ fore ends contained progressively smaller numbers of glycogen inclusions. The + and ++ fore-end curves are separated by a relatively large interval. Because the curves in *A* are considered to represent in general the effects of administration of each of the arsenical poisons used in this work, the curves for each poison are not presented. The curves for paris green (*B*), however, indicate a progressive decrease in number of blood-cell glycogen inclusions as the general hematological changes progress to the +++ stage.

The larvae receiving calcium arsenite had fore ends showing only +++ conditions and hind ends in which slight hematological changes tending toward the + condition usually seemed to occur. One larva had a hind end showing a ++ condition. The

larvae given calcium arsenate had hind ends showing 0 and fore ends showing only + and ++ changes.

The glycogen counts for larvae given arsenic trioxide and lead arsenate present a graphical picture similar to those of figure 5.

A comparison of average hind-end and fore-end counts (not illustrated) for barium fluosilicate, sodium fluoaluminate, and sodium fluoride showed that, as was true for the arsenical curves, the numbers of blood-cell glycogen inclusions in classes 1 to 3+ for the hind ends tended to exceed those for the fore ends. Figure 6, *A*, corresponding
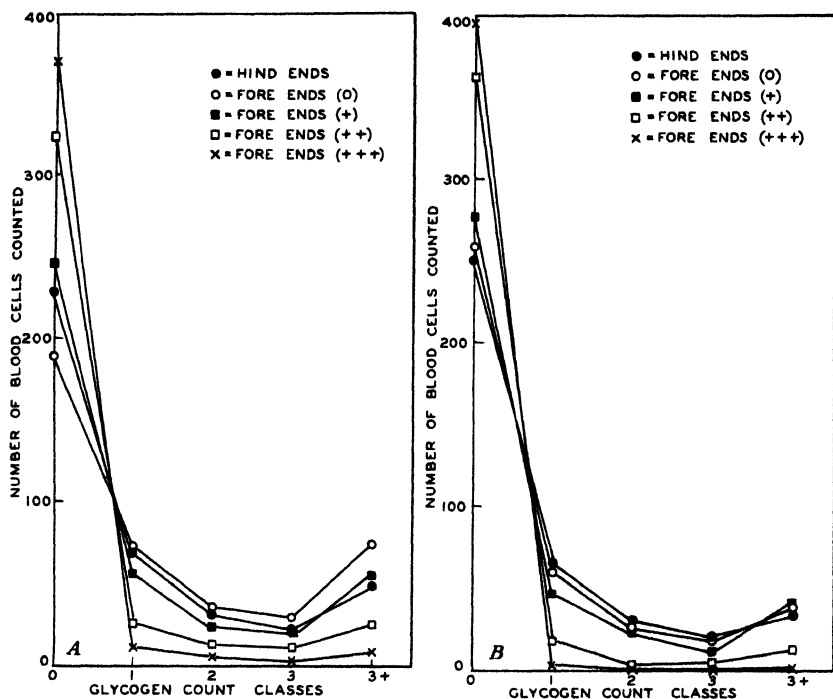
FIGURE 5.—Average glycogen counts from hind and fore ends of larvae fed carbohydrate-turnip-leaf sandwiches, ligatured, and then given poison in corn-starch-turnip-leaf sandwiches, the fore-end counts being grouped according to whether they showed a 0, +, ++, or +++ stage of hematological change: *A*, Larvae poisoned with Paris green, lead arsenate, calcium arsenite, calcium arsenate, and arsenic trioxide; *B*, larvae poisoned with paris green.

to figure 5, *A*, shows the average glycogen curves for the combined hind ends and for the combined 0, +, ++, and +++ fore ends of the larvae given these poisons. Here also the hind-end and 0 and + fore-end curves, and the ++ and +++ fore-end curves tend to fall into two groups, which are separated by a relatively large interval. Among the curves for the individual poisons, this grouping was most marked when sodium fluoaluminate was used. When barium fluosilicate was used, the grouping was slight and the relatively large interval was between the 0 and + fore-end curves (fig. 6, *B*). These curves indicate a progressive decrease in the number of blood-cell glycogen inclusions of classes 1 to 3+ as the hematological changes progress. Because figure 6 is considered to represent the general

effects of administering the fluoride poisons used in this work, the curves for sodium fluoaluminate and sodium fluoride are not presented.

Figure 7 contains average-glycogen-count curves for the hind ends and for the 0, +, ++, and +++ fore ends of the larvae given mercuric chloride. The curves indicate a progressive decrease in number of blood-cell glycogen inclusions as the hematological changes progress. As with the arsenical and fluoride curves, a tendency toward grouping is shown. The value for the 3+ class is relatively

FIGURE 6. Average glycogen counts from hind and fore ends of larvae fed carbohydrate-turnip-leaf sandwiches, ligatured, and then given poison in cornstarch-turnip-leaf sandwiches, the fore-end counts being grouped according to whether they showed a 0, +, ++, or +++ stage of hematological change: *A*, Larvae given sodium fluoaluminate, sodium fluoride, and barium fluosilicate; *B*, larvae given barium fluosilicate.

high in the ++ fore-end curve. Similarly, relatively high values of the 3+ class occur in the + fore-end curves of figure 5.

Average glycogen counts for the nonligatured larvae subjected to nicotine vapor for periods up to 29.5 hours and for the corresponding control larvae are represented in figure 8. The two curves are of the same form and show no marked differences.

All the average curves, such as those shown in figures 2 to 8, have the form $0 > 1 > 2 > 3 < 3+$.

DISCUSSION

METHODS

The complicating factor of individual differences between larvae was eliminated by the use of ligatured insects, because blood from the

fore end could be compared with blood from the hind end of the same individual. Any hematological change resulting from the ingestion of a poison by an adequately ligatured larva can be expected to appear only in blood from its fore end, whereas blood from the hind end may be used as a control. A differential effect upon the blood that might be produced by ligaturing should be apparent from a comparison of fore-end and hind-end blood taken from a ligatured but unpoisoned larva. A poison coming in contact with the integument of either end may be expected to occur in and possibly to affect the blood in that end, provided the integument is permeable to the poison.
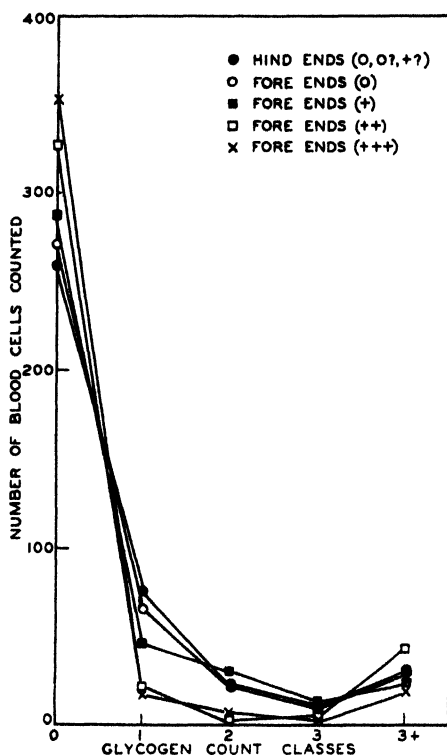
FIGURE 7.—Glycogen counts from the hind and fore ends of larvae fed carbohydrate-turnip-leaf sandwiches, ligatured, and then given mercuric chloride, or mercuric chloride in cornstarch-turnip-leaf sandwiches, the fore-end counts being grouped according to whether they showed a 0, +, + +, or + + + stage of hematological change. The + and + + fore-end curves represent single counts; the other curves, average counts.

Several investigators have reported that some insecticides can penetrate the integuments of some insects. For example, integumental absorption of arsenic has been reported by O'Kane and Glover (*17*), Lepesme (*14*), and Woodworth (*27*); of fluoride by Hockenyos (*10*); of pyrethrum by Wilcoxon and Hartzell (*25*); and of nicotine by Richardson, Glover, and Ellisor (*21*). The nicotine vapor possibly entered the insect's body partly by integumental absorption and partly through the tracheal system. Kitchel and Hoskins (*11*) and McGovran (*16*) have shown that in nicotine vapor (of lower concentrations

than used here) tracheal ventilation of the cockroach and of the grasshopper does continue.

In most of the present experiments the larvae were free to bring the integument of either the fore or the hind end in contact with the administered poison, but in several experiments they were placed in stocks (pl. 1, *B*) to prevent contact of the hind end either with the fore end or with the poisoned sandwich. Although some integumental absorption probably occurred, for example, when calcium arsenite was used, it is considered that the poison entered the larva's body chiefly by ingestion and intestinal absorption, because of the occurrence in some of the smears of abnormal midgut epithelial cells, some of which were much vacuolated, large numbers of bacteria, and fragments of partly digested leaf. Parfentjev (*18*), Pilat (*19*), and
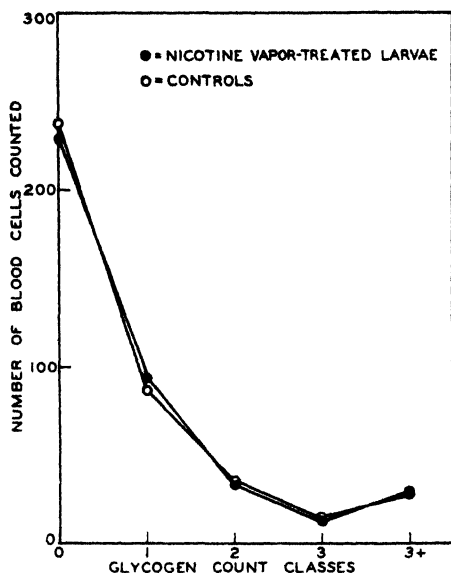
FIGURE 8.— Average glycogen counts from nonligatured larvae fed carbohydrate-turnip-leaf sandwiches and then treated with nicotine vapor for periods up to 29.5 hours, and from their controls.

Woke (*26*) have demonstrated that ingestion of arsenicals and fluorides may be followed by necrosis and loss of midgut epithelium. The poisoned and unpoisoned sandwiches were like those used by Campbell and Filmer (*2*), except that the sandwiches used here contained cornstarch, or cornstarch and sugar, and an unmeasured, relatively large amount of poison. Ligatured insects have been used by Fraenkel (*7*) in an investigation of hormones in *Calliphora* and by Ermakov (*3*) in a study of the reducing power of silkworm blood.

Comparison of blood smears from fore and hind ends of unpoisoned larvae indicated that blood-cell glycogen tended to become relatively lower in the hind end, particularly in larvae that had been ligatured for the longer periods (pl. 3, *F*, and negative value of M_{H-F} in table 1). Although this difference might result from an actual increase in the fore end, a decrease in the hind end, or certain combinations of changes in both ends, it is probably due partly to a real decrease in

the hind end, because microscopic observations indicated that prolonged ligaturing may induce degenerative changes sooner in this end. In some experiments hind-end blood smears showed a + or, in several larvae, even a + + effect, particularly with calcium arsenite, mercuric chloride, and arsenic trioxide. These poisons must therefore have penetrated into the hind end of the larva either by absorption through the integument or by diffusion from the fore end through the ligatured region.

The glycogen counts and indices are based upon 400 cells taken at random, since this number could be checked satisfactorily for the purpose of this work and it is the same as that recommended by Kolmer and Boerner (*12*, *p. 85*) and given by Kracke and Garver (*13*, *p. 480*) for bloods with total counts of 20 to 25 thousand cells per cubic millimeter.[4]

GENERAL HEMATOLOGICAL CHANGES AND PASSIVE-ACTIVE TRANSFORMATION

For a more complete understanding of the initial changes in the blood cells of an insect subsequent to poison administration, it is necessary to consider the form changes that blood cells of many insects undergo under certain physiological conditions. Tait (*23*) and Loeb (*15*) observed several types of blood coagulation in Crustacea. Yeager and Knight (*30*) observed similar kinds of blood coagulation in a number of different insects. Coagulation of insect blood was found to involve a plasma coagulation, a cell coagulation or agglutination, or both. In a study of the coagulation of blood from the oriental cockroach, Yeager, Shull, and Farrar (*32*) found that during this process the blood cells may lose their original fusiform or discoid shape, round up, become more refractive, form pseudopodia, agglutinate into a number of clumps, spread out on supporting surfaces, and seemingly disintegrate. They also reported that heat fixation of the roach at 60° C. for 5 to 10 minutes prevents subsequent coagulation and changes in form of the blood cells. Ermin (*4*) also has recently studied the changes undergone by coagulating insect blood cells.

Marked change in form of blood cells during coagulation has long been recognized as characteristic of invertebrate blood (*8*, *9*, and others cited in *5*, *4*, and *22*). Unpublished observations by the present authors have shown that coagulation of the blood of *Prodenia eridania* involves marked changes in form of several types of blood cells, including the plasmatocytes and the cystocytes. Thus, at least certain types of insect and other invertebrate blood cells are capable of existing in two different forms. It may be considered that such a blood cell tends to assume one form—for example, a spindle shape—when suspended in the circulating blood plasma in a quiescent state, unaffected by exciting stimuli, but when affected by exciting stimuli to undergo changes such as withdrawing its spindle ends, tending to round into a spheroid or ovoid, producing irregularities in its surface protoplasm, extending some type of pseudopodium or lamella, or tending to form a very thin cell, widely spread upon a surface. Through some such change a cell having a passive form and suspended in the circulating blood plasma may develop an active form and display some kind of cellular activity. Such activity may involve the

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[4] Counts made by the senior author and J. B. Gross indicate that the average value for the mature larva of *Prodenia eridania* is about 20 to 25 thousand cells per cubic millimeter of blood.

formation of pseudopodia, with or without actual locomotion, cellular locomotion, capsule formation, possibly phagocytosis, etc.

Fauré-Fremiet (5) made an extensive study of passive and active states of blood cells of a number of marine invertebrates other than insects, giving careful descriptions of each state and of the passive-active transformations undergone by various kinds of cells. The present authors have observed insect blood cells, in the active state that are analogous to some of the active forms described by Fauré-Fremiet; these observations were made particularly upon blood from *Prodenia eridania* and *Periplaneta americana*. The senior author has further observed such passive-active transformations and active forms in several other species of insects (30).

A study of the plasmatocytes and cystocytes in the blood smears showing a + hematological change obtained from larvae given the arsenicals, the fluorides, and mercuric chloride indicated that one of their earliest changes is from a passive to an active form. In fact, the chief characteristics of the + stage of general hematological change can be explained to a great extent on the basis of passive-active transformation and blood-cell coagulation. The slight tendency of some of the blood cells from larvae given noneffective poisons to deviate from the normal was exhibited chiefly as partial passive-active transformation. The + stage, therefore, is considered to represent a certain physiological state of the blood which might be, but is not necessarily, accompanied by a toxicological condition of the blood cells. This physiological state of the blood is characterized by the partial or complete transformation of many of the plasmatocytes and cystocytes and an increase of blood-cell agglutination in vivo. In smears from presumably normal larvae there is often a slight, variable amount of blood-cell agglutination.

The ++ stage of general hematological change involves the changes characteristic of the + stage, explainable for the most part on a physiological basis and not necessarily toxicological as regards the blood cells. It also involves a more marked agglutination, some distortion, and perhaps some disintegration, all of which, particularly the distortion and disintegration, may represent a toxicological or a pathological disturbance of the affected cells.

The +++ stage differs from the ++ stage chiefly in the greater amount of cell distortion and disintegration, or, in other words, in a greater toxicological disturbance of the blood cells, which frequently appeared in obviously smaller numbers during this stage. The decrease in numbers of cells could result from a complete disintegration of some of the affected cells or from a dropping out of the circulating blood of clumps of agglutinated cells or of single cells. In invertebrate blood coagulation, during which certain cells undergo passive-active transformation, their surfaces undergo physicochemical changes resulting in increased stickiness (4, 5, 15, 23, 32). The cells adhere to one another and tend to form agglutina, which can be caught among various tissues and thus removed from the circulating blood. The active blood cells also tend to adhere to certain surfaces, including those of injured tissues.[5] It is thus quite possible that blood cells disappeared from the circulating blood through cellular degeneration, through adhering to certain injured tissues in

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[5] Such adhesion of the blood cells to the tissues at the site of injury can be demonstrated by observing the circulating blood in the wing of a cockroach (28 and 29) and thrusting a needle through the wing.

the poisoned portion of the insect's body, and through the removal of cell agglutinin from circulating blood to tissues. The observation that blood cells tend to decrease especially in the $++$ and $+++$ stages of general hematological change is in agreement with the report of Fisher (*6*) that a decrease in the total blood-cell count of the oriental cockroach occurs subsequent to the administration of lethal doses of white arsenic, sodium fluosilicate, and mercuric chloride.

RELATION OF GLYCOGEN DECREASE TO GENERAL HEMATOLOGICAL CHANGES

The glycogen-count curves in figures 5 to 8 and the glycogen-index values given in table 2 indicate that, in general, blood-cell glycogen shows little or no decrease in the $+$ stage and the greatest decrease in the $++$ and the $+++$ stages. This observation is in accord with the view that the $+$ stage represents largely a physiological condition of the blood, whereas the $++$ and $+++$ stages represent predominantly toxicological conditions.

CYTOLOGICAL CHANGES FOLLOWING POISON ADMINISTRATION

POSSIBLE REGENERATIVE CHANGES

The only indication of regenerative changes noticed in these experiments was an apparent tendency of the nuclei of some cells to exhibit mitotic, particularly prophasic, aspects. Some of these nuclei were obviously in a mitotic condition, but many were questionably so. The mitotic cells seemed to be unusually numerous, but as the microscopic observations were only qualitative an increase of these cells was not definitely demonstrated. The possibility of the occurrence of such regenerative changes is stressed here because similar observations of an apparent increase in mitosis among blood cells from poisoned insects have been reported by Pilat (*20*), Lepesme (*14*), and Tareeva and Nenjukov (*24*). None of these authors reported quantitative mitotic counts. Further investigation of a more quantitative character appears to be necessary to demonstrate definitely an increased rate of mitosis in insect blood subsequent to poison administration.

DEGENERATIVE CHANGES

Observations on the blood cells from poisoned insects have been made by Pilat, Lepesme, and Tareeva and Nenjukov. Pilat (*20*) studied the blood cells of *Locusta migratoria* that had been poisoned with sodium arsenite and sodium fluosilicate (silicofluoride). Although he considered that poisoning produced degeneration of some blood cells, Pilat concluded that the blood picture of the poisoned insect is too complicated to be quantitatively expressed until precise and uniform principles of blood-cell classification are established. Tareeva and Nenjukov (*24*) reported degeneration among the blood cells of *Calliptamus* given sodium arsenite. Cellular degeneration apparently involved achromophilia, cytoplasmic fragmentation and disintegration, nuclear fragmentation and disintegration, and reduction of nuclear-cytoplasmic differentiation. Lepesme (*14*), in a study of the penetration of arsenicals through the integument of *Schistocerca gregaria* Forsk., found that blood cells from poisoned insects exhibited

cytoplasmic vacuolization, loss of cellular contours, and disintegration of the chromatin masses.

None of these authors studied the effects of poison administration upon the blood cells of Lepidoptera, they used different hematological techniques from those used in the present experiments, and they did not consider the possibility of passive-active transformations among the blood cells that they studied. Their descriptions can therefore be compared only in a general way with observations reported here for *Prodenia eridania*, but there is agreement in indicating that administration of the arsenicals and fluorides can produce degeneration of blood cells in insects.

With regard to degenerative changes, the present observations on the blood of poisoned larvae of *Prodenia eridania* show not only that different types of cells may respond differently to the administration of one of the effective poisons, but also that the arsenicals, the fluorides, and mercuric chloride may produce somewhat different effects upon a given type of blood cell. They also indicate that blood-cell degeneration involves several kinds of cytological change, among which are plastid formation, achromophilia, partial or complete loss of cytoplasmic structure, disappearance of glycogen inclusions (with or without an accompanying diffuse glycogen stain (Bauer)), disruption of nuclear structure, and nuclear extrusion. Plastid formation may be a manifestation of a pronounced or abnormal pseudopodial activity. The disappearance of cytoplasmic structures and the presence of a diffuse glycogen stain coincident with the disappearance of glycogen inclusions may indicate a dissolution of these structures and of glycogen.

EFFECTS OF ADMINISTRATION OF DIFFERENT POISONS ON CYSTOCYTES AND PLASMATOCYTES

The scheme shown in figure 9 has been devised to illustrate in a general way the changes undergone by the plasmatocytes and the cystocytes subsequent to the administration of the effective poisons. It indicates that during the + stage of general hematological change the affected plasmatocyte or cystocyte tends to change from its passive to its active form. In their active form some of these cells possess smooth surfaces and may appear swollen, while others may not appear swollen but may exhibit various surface irregularities, involving the ectoplasm. Thus far these changes can be considered to be physiological rather than toxicological, with the possible exception of the swollen aspect, which may be abnormal. The questionable occurrence of regenerative, mitotic changes during the + or ++ stage is indicated. To what extent these changes and the final assumption of all the different cellular forms that have been called active can be considered to be physiological or toxicological is problematical. The degenerative changes, which may be considered toxicological, occur chiefly in the ++ and +++ stages. During these changes blood-cell glycogen decreases or disappears. Cellular degeneration may or may not involve plastid formation. The various details previously presented and discussed are implied by this scheme, but they have not been actually represented.
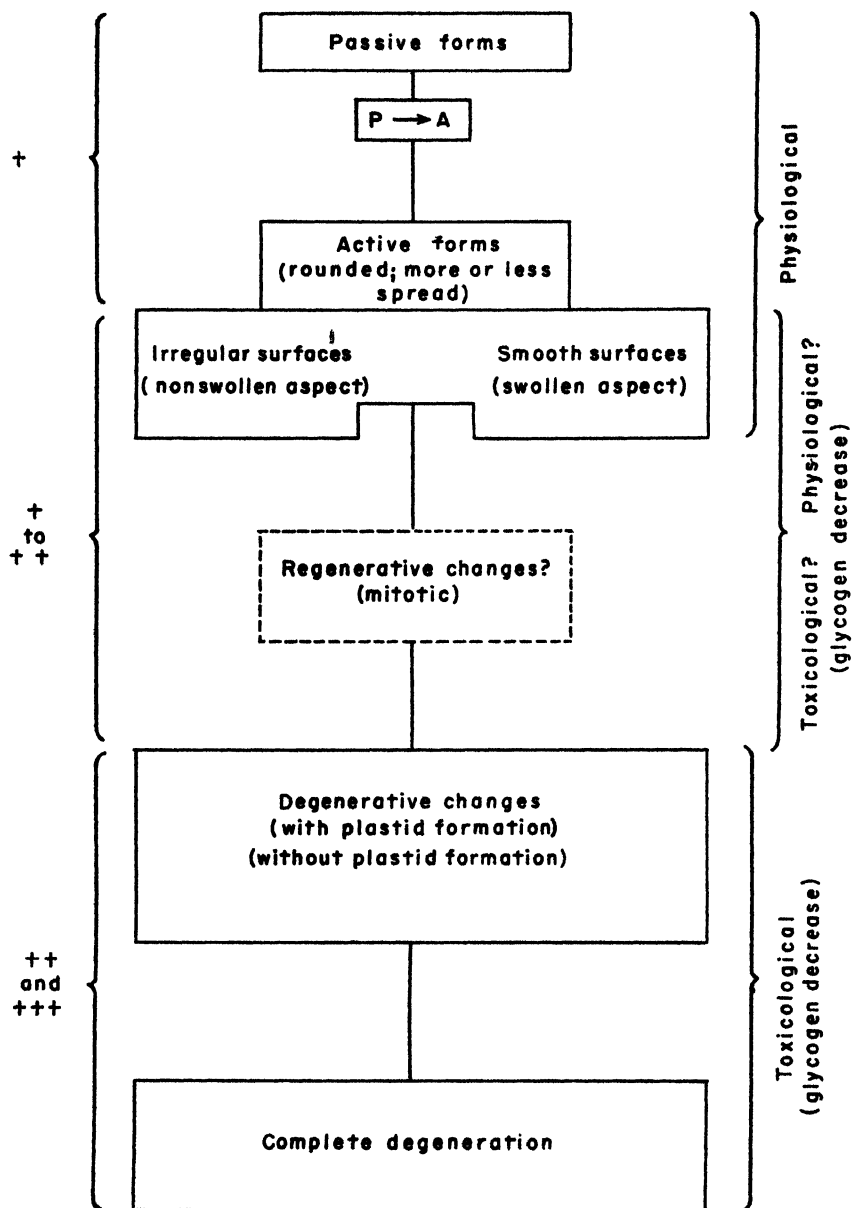
FIGURE 9.—A generalized scheme illustrating the hematological changes observed to follow administration of the arsenical and fluoride poisons and mercuric chloride.

POSSIBLE CHANGES AFTER ADMINISTRATION OF THE OTHER POISONS

Although no obvious cytological changes followed administration of nicotine peat, nicotine bentonite, and rotenone, occasional blood cells looked as though they might be just beginning to undergo pas-

sive-active transformation. Exposure to nicotine vapor up to 6 hours was followed by no obvious alteration of the blood cells, except perhaps a slight tendency of some of them to round up, but after exposure for prolonged periods (23 hours or more) the blood cells showed a definite tendency toward passive-active transformation and an abnormal amount of vacuolization (pl. 2, *B*). Whether these changes were produced directly by the nicotine vapor or by an accompanying alteration in the insect's body, such as dehydration, was not determined. The fact that the dehydrated controls did not show the same degree of change suggested that with these long exposures the blood cells were somewhat affected, either directly or indirectly, by nicotine vapor. The failure of nicotine vapor readily to produce marked changes in the blood cells is in agreement with the results of Babers (*1*), who similarly exposed *Prodenia eridania* larvae to concentrated nicotine vapor and found that the pH of the blood was maintained at the normal value of approximately 6.65. When pyrethrum was used, the blood cells showed some indications of passive-active transformation. In general, with these exceptions, administration of the non-effective poisons produced no obvious cytological changes in the blood cells.

MECHANISM OF POISON EFFECT

Reference has been made to the effects of poison administration rather than to the effects of poison on blood cells. Although it is probable that at least those degenerative cytological changes and the blood-cell glycogen decrease occurring in the $++$ and $+++$ stages were caused by direct action of the poison in the plasma, proof of this occurrence is not given by the results of this investigation. These results do indicate that administration of the effective poisons caused a decrease of blood-cell glycogen as a part of a general cellular degeneration rather than in a specific manner.

SUMMARY AND CONCLUSIONS

Ligatured larvae whose blood-cell glycogen had previously been increased by ingestion of turnip-leaf-cornstarch-glucose sandwiches were given access to turnip-leaf-cornstarch sandwiches without poison (controls) and turnip-leaf-cornstarch sandwiches containing poison. The poisons used were nicotine bentonite, nicotine peat, rotenone, pyrethrum, phenothiazine, calcium arsenite, calcium arsenate, arsenic trioxide, paris green, lead arsenate, sodium fluoride, sodium fluoaluminate, barium fluosilicate, and mercuric chloride. Blood smears from the fore and hind ends of the poisoned ligatured larvae were stained for glycogen. From a study of these smears results were obtained leading to the following conclusions:

(1) No marked hematological changes followed the administration of nicotine bentonite, nicotine peat, rotenone, pyrethrum, and phenothiazine, although some of the fore-end blood smears from larvae given pyrethrum showed slight changes consisting largely of partial passive-active transformation. To what extent this lack of effect was caused by failure of the administered poison to enter the blood stream was not determined.

(2) Administration of none of the poisons named under (1) caused a significant decrease of mean glycogen index of the fore ends relative to that of the hind ends of the treated larvae, but relative decreases

in fore-end glycogen indices did occur subsequent to administration of the arsenicals, the fluorides, and mercuric chloride.

(3) Marked hematological changes in fore ends relative to hind ends of the ligatured larvae followed the administration of the arsenicals, the fluorides, and the mercuric chloride.

(4) The hematological changes that followed poison administration occurred progressively and, for purposes of description, are grouped according to successive stages as 0, +, + +, and + + +, where 0 presents the normal and + + + the stage of most marked change. The + stage is characterized chiefly by partial or complete passive-active transformations (especially rounding up) of certain blood cells, including the cystocytes and the plasmatocytes, and by a slightly augmented cell agglutination. It is thus considered to represent chiefly physiological changes, which may or may not be accompanied by the beginning of certain toxicological changes. The + + and + + + stages are characterized chiefly by more marked agglutination, distortion, disintegration, and apparent loss of cells from the blood. In general, subsequent to the administration of the arsenicals, the fluorides, and mercuric chloride, the earlier blood-cell changes (+ stage) tended to be chiefly physiological, such as may occur in larvae not subjected to poison administration, whereas the later changes tended to be chiefly toxicological (or pathological).

(5) An increase of mitosis seemed to occur soon after administration of the arsenicals, the fluorides, and mercuric chloride, but was not demonstrated quantitatively.

(6) The degenerative cytoplasmic changes consisted of apparent cellular swelling, disruption of and decrease in visibility of normal structure, achromophilia, decrease or loss of blood-cell glycogen, formation of broad pseudopodia or cytoplasmic bulges, plastid formation, excessive vacuolization, and raggedness. Nuclear degeneration involved distortion, raggedness, loss or disruption of normal structure, achromophilia, assumption of more or less peripheral position, fragmentation, pycnosis, and extrusion.

(7) Subsequent to mercuric chloride administration the nuclei of certain cystocytelike cells gave a positive reaction to the Bauer test for polysaccharide; this reaction was not observed after the administration of the other poisons.

(8) Plastid formation tended to follow arsenical and mercuric chloride administration more readily than fluoride administration.

(9) Abnormal cellular vacuolization (gross vacuoles) tended to follow administration of the fluorides and, especially, of mercuric chloride.

(10) Decreases of glycogen index subsequent to arsenical, fluoride, and mercuric chloride administration tended to occur to a greater extent during the + + and + + + stages of general hematological change than during the + stage. Thus, the decrease of blood-cell glycogen is considered to occur as a part of cytological degeneration and not as a specific, exclusive effect of poison administration.

(11) As blood-cell glycogen decreased subsequent to poison administration, the glycogen count tended to show a general decrease in the classes 1 to 3+, inclusive, and to maintain the normal form of $0 > 1 > 2 > 3 < 3+$.

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THE RELATION OF SHANK COLOR TO WINTER EGG PRODUCTION CHARACTERISTICS OF SINGLE-COMB WHITE LEGHORN PULLETS [1]

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INTRODUCTION

The belief that heavy egg production is accompanied by the bleaching of pigmented structures in yellow-skinned breeds had been held by practical poultrymen long before the first quantitative data on this correlation were presented by Blakeslee and Warner (*2, 3*).[3] A series of experiments led Palmer and Kempster (*10*) to suggest that xanthophyll from the feed, instead of being deposited in the ear lobes, beak, shank, eye ring, and the skin surrounding the vent, as it is in males and in nonlaying females, is diverted in laying birds to the forming ova. The epidermal xanthophyll in the meantime "gradually disappears as the result of the natural physiological change in the structure of the skin." Cessation of production is followed by return of xanthophyll to the parts named. These phenomena have enabled poultrymen to estimate the past production of birds from the degree of bleaching or the extent of return of pigment to these tissues.

The quantitative data on the relation between pigmentation and past production presented by various investigators seem to be largely confined to those gathered toward the end of the laying year. Thus Blakeslee and his collaborators (*1, 4*) found the correlation coefficients between pigment in the ear lobe (determined by matching with a color top) in October of the second year of life and the annual production in two groups of White Leghorns to be -0.5816 ± 0.0253 and -0.5271 ± 0.0252, respectively. By analyzing the relationship of the pigmentation observed in October with the monthly production in the course of the previous year, these investigators concluded that the correlation was largely due to the production during the month immediately preceding the date of observation. Warner (*13*) extended this study to include pigmentation changes in the color of the vent. He also noted that the correlation exists not only at the end of the year, but also at the beginning and middle of the year. Sherwood (*11*), who made observations in September, likewise found his subjective grading of shank and beak color to be correlated with annual production, the coefficients of correlation being of the magnitude of -0.6. Similar results on the basis of subjective color determinations were obtained by Hervey (*5*) and by Knox and Quinn (*7*).

In a recent textbook, Jull (*6, p. 431*) suggested that observations on the degree of bleaching of the beak and shank in December or January of the first year of life make it possible to estimate the in-

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herent rate of production of individuals thus examined. If the theory of Palmer and Kempster (*10*) is valid, however, pigmentation changes at that time may reflect not only the rate of production but also the length of the preceding laying period, and thus include maturity and winter pausing. Since no work has been reported on the relation of pigmentation changes to the particulate factors of egg production, it appears to be desirable to examine whether the bleaching of the pigmented structures of yellow-skinned pullets depends on the length of the preceding laying period, on the rate of production, or on both.

MATERIAL AND METHODS

The University of California flock of Single-Comb White Leghorn pullets, consisting of birds bred for production as well as for other characteristics, was used for this analysis. All of the pullets alive at the time the observations were made were used. The shank was the only pigmented structure considered.

TABLE 1.—*Composition of standards used in determining shank color*

	Percentage composition		
Shank color grade	Plaster of paris	Chrome yellow	
		Intermediate orange grade	Yellow grade
1	95	5 0	0 0
2	90	10 0	0
3	85	15. 0	. 0
4	80	17 5	2 5
5	75	20 0	5 0
6	70	22. 5	7 5
7	65	25 0	10 0
8	60	27 5	12 5
9	55	30. 0	15 0
10	45	32 5	22 5

In order to obtain objective measurements of color, a color comparator was used. The comparator consisted of a series of 10 test tubes mounted on a wooden block and containing a mixture of plaster of paris and chrome yellow in different proportions. The changes in the value of the mixture from grade to grade were linear in order, so far as the percentage of white was concerned, except for the transition from grade 9 to grade 10. The range of hue and purity used in the comparator were based on a study of the color constants of actual shanks. Table 1 presents the composition of the standards. The colors used approximate those represented in the upper half of plate 9 of the color dictionary of Maerz and Paul (*9*), running from 2 to 6 on the horizontal and from C to L on the vertical scale. The upper third of the back of the shank of each bird studied was matched against the standards under comparable light conditions for all the birds.

The grade was determined jointly by the writer and H. B. Mugglestone, who assisted in making the observations. The degree of reliability of the grades assigned may be judged by the fact that in a preliminary test on 26 birds, the independent replicate grades showed a correlation coefficient of 0.937 ± 0.024 with the original grades. Birds

were weighed at the same time that shank-color determinations were made. To eliminate age as a variable, the 4 hatches of Single-Comb White Leghorn pullets used in the study were examined about a week apart. These birds were hatched in March and April 1940, and the color and weight observations were made between November 26 and December 17, at about 8 months of age. The total number of birds involved was 969. Of these, 39 had not started laying at the time the observations were made. Their mean shank-color grade was 6.62 ± 0.48, as compared with 3.85 ± 0.07 for the remaining 930 birds. Since production characteristics for these 39 birds were not available, they were omitted from the part of the analysis in which egg-production characteristics were employed.

The color of the shanks preceding initiation of laying of the 930 pullets used in this analysis was not available. Since considerable variation undoubtedly existed with respect to the original intensity of pigmentation in these birds, it may be argued that the observations recorded here do not reflect pigmentation changes but rather represent shank color at the given age. In practice, however, such single examination is the procedure used for evaluation of past production as indicated by the cited suggestion of Jull. Hence, while the precision of the term "pigmentation changes" may be debatable, the observations made nevertheless permit the estimation of the variation in shank color and indirectly in the degree of bleaching.

The present study is not concerned with the nutritional aspects of the problem. It should be noted, however, that the birds of each hatch were divided evenly into two groups at the time of hatching, one group being fed a ration containing about 50 percent of yellow corn, the other a ration in which about 30 percent of barley was substituted for the equivalent amount of corn. When the birds were 5 months of age the second of the rations mentioned was fed to both groups. Fresh greens were supplied approximately every other day to all the birds.

DEFINITION OF TERMS

For the purposes of the following discussion, the terms to be used are defined as follows:

Shank color. - The colorimetric grade assigned to each bird in accordance with the procedure outlined above.

Maturity. - The number of days from hatch to date of the first egg.

Production. - The number of eggs laid by each bird from the first egg to the date on which observations on shank color were made. This is roughly the production to 8 months of age.

Length of laying period. - The number of days elapsed from the date of first egg to the date of shank-color observations.

Days in pause. - The cumulative length of pauses, each period of 7 or more consecutive nonlaying days after maturity being considered a pause.

Days in production. - The length of laying period less days in pause.

Body weight. - The weight of the bird in grams on the day of shank-color observations (roughly at 8 months of age).

Gross rate. - The production times 100 divided by days in production.

Net rate. - The production times 100 divided by active laying days. The distinction between gross and net rate has been discussed in detail by Lerner and Taylor (8).

DATA AND DISCUSSION

The means and standard errors of the variables studied are presented in table 2. Although the birds in the different hatches were

examined at approximately the same age, the shank color showed an increase from the earliest to the latest hatch. This progression is not linear. It may be associated with the later maturity of the birds hatched later in the season. The seasonal factor is also in evidence with respect to body weight, the later hatched birds being smaller than the earlier hatched ones at the same age. It is likely that the definitive adult weight has been approached more closely by 8 months of age in the earlier hatches.

TABLE 2.— *Means and standard errors of the variables studied* [1]

Variable studied		Hatch No 1, 217 birds	Hatch No. 2; 217 birds	Hatch No. 3; 210 birds	Hatch No. 4, 286 birds	All hatches. 930 birds
Shank color	grade	3 17±0 13	3 60±0 15	3 60±0 13	4 73±0 15	3 85±0 07
Production	eggs	51.6±1 5	50 6±1.6	52 2±1.5	45.7±1 3	49.7±0.7
Length of laying period	days	81.3±1.8	84 7±1.8	80 3±1 6	76.1±1.5	80.3±0.8
Days in production	do	70 6±1.9	72.1±1 9	73 2±1.8	65.2±1 7	69.9±0 9
Body weight	grams	1,719±14	1,658±16	1,660±16	1,601±14	1,655±8
Maturity	days	169.4±1 7	172 1±1 7	173 5±1 6	178 6±1 5	173.8±0 8
Net rate	percent	71 8±0 8	68.5±0.8	70 7±0.8	70 0±0.8	70.2±0 4
Gross rate	do	63 7±1 3	59 4±1 3	64 5±1.3	59.8±1 2	61.7±0.7
Days in pause	days	10.7±1 4	12.6±1.4	7 1±1.1	10 9±1.3	10.4±0 6

[1] The slight discrepancies between the actual derived variables (days in production, net and gross rate) and the various ratios of the primary variables (production, length of laying period, days in pause) which lead to the calculation of the derived variables from the tabular means, are due to rounding off of decimals in the calculations

Table 3 presents the coefficients of correlation between shank color and the other variables studied for each hatch separately, and for the group as a whole. The latter coefficients are shown as computed from the ungrouped population (observed) and as obtained by the z_r transformation (calculated) from the individual values in the four hatches (*12*). The closeness of the observed and the calculated values would suggest that the former can be safely used as the best estimate of the magnitude of the correlation in the population as a whole. There are two possible departures from this: (1) The correlation of shank color with body weight increased significantly from the first to the fourth hatch, and (2) the correlation with net rate was lower in the last two than in the first two hatches. It is doubtful whether any significant conclusion can be derived from the latter decrease. So far as the shank color-body weight relationship is concerned, it is possible that the preproduction correlation between these two variables is high and is gradually reduced as the shank color bleaches.

TABLE 3.—*Coefficients of correlation of shank color with variables indicated*

Variable	Hatch No. 1	Hatch No. 2	Hatch No. 3	Hatch No. 4	All hatches Observed	All hatches Calculated
Egg production	−0 503	−0 612	−0.548	−0.463	−0 529	−0 53
Length of laying period	−.241	− 390	−.298	−.313	−.326	−.31
Days in production	−.479	−.611	− 518	−.480	− 521	−.52
Body weight	− 055	026	.184	.549	.173	.19
Maturity	.235	.381	.287	.305	328	31
Net rate	−.339	−.352	−.179	−.069	−.209	− 24
Gross rate	−.480	−.471	−.494	−.353	−.432	−.45
Days in pause	.375	.359	.407	.284	.329	.36
r at $P = 0.05$	.138	.138	.138	.113		
r at $P = 0.01$	.181	.181	.181	.148		

Thus the shank color in hatches 1 and 2 is independent of body weight, whereas a high correlation is still shown in hatch 4.

The highest correlation coefficients observed were between shank color on the one hand and egg production and days in production on the other. Since these two variables are themselves highly correlated, it is possible that one or the other is only indirectly related to shank color. An analysis of partical correlation coefficients, which are presented in table 4, yields an answer to this question.

TABLE 4.—*Coefficients of partial correlation between shank color and the variables indicated*

Variable correlated with shank color	Variables eliminated	Hatch No. 1	Hatch No 2	Hatch No. 3	Hatch No 4	All hatches
Egg production	Length of laying period	−0.472	−0 514	−0 492	−0 359	−0 442
	Body weight	− 507	− 619	− 581	− 617	− 554
	Maturity	−.477	−.520	− 509	−.365	− 441
	Net rate	−.440	− 536	−.533	−.464	− 498
	Days in pause	− 394	− 539	− 439	− 385	− 447
Length of laying period	Egg production	.144	037	.115	− 009	045
Maturity	do	− 154	−.041	−.173	013	− 041
Net rate	do	−.097	017	.099	080	.040
Days in pause	do	.173	.102	194	064	090
Egg production	Maturity / Net rate / Days in pause	.104	− 003	− 184	112	− 034

It may be seen that when the effect of egg production is eliminated, the length of the laying period is no longer significantly correlated with shank color. Under the same condition shank color is not significantly correlated with days in pause.[4] Since the days in production are obtained by the subtraction of days in pause from the length of laying period it may be seen that egg production is the primary correlative of shank color.[5] However, egg production itself is determined by maturity, net rate, and days in pause. When the effect of any one of these is eliminated, egg production still shows a high correlation with shank color. Conversely, the elimination of egg production in the partial correlation of each of these variables with shank color reduces the respective coefficients to nonsignificant levels. Yet the third-order partial correlation between egg production and shank color, with the effects of maturity, net rate, and days in pause eliminated is practically zero. It thus appears that the combined action of these three factors acting through egg production is the significant determinant of the changes in shank color. Individually, their contribution is low; combined they account for 27.98 percent of the variance in shank color. The multiple determination of shank color, with egg production and body weight as the independent variables, increases this figure to 32.74 percent.

This, of course, means that shank color as a criterion of production cannot be used for independent estimation of the component factors of egg production, such as rate, as suggested by Jull (6). It

[4] It may be noted in passing that once more no correlation was found between net rate and length of winter pause. The correlation coefficient for the combined population was −0.091, confirming the suggested independence of these variables (8).

[5] It is possible that the significant factor determining depth of pigmentation is the length of time that the birds are in production without pauses of sufficient length to permit deposition of pigment. If this is true, then rate would have no effect unless birds are capable of laying very slowly and at the same time deposit pigment in the shanks. There is, however, no evidence that such is the case. It is unfortunate that because of the intimacy of the correlation between production and days in production this alternative cannot be subjected to verification. Should this idea be valid it only lends further support to the main conclusion that rate of production cannot be evaluated by pigmentation changes.

is an exceedingly valuable tool in culling practice for identification of low or nonproducing individuals. In flock-testing procedures, however, observations on shank color cannot replace trap-nesting, if information on the component factors contributing to the egg record is desired.

SUMMARY

An analysis of the relation of shank color at 8 months of age to production factors in a flock of 930 Single-Comb White Leghorns indicates that:

(1) Differences in egg production account for about 28 percent of the variance in shank color.

(2) This relationship is due to the combined effect of maturity, rate, and pause, rather than to any of these variables alone.

(3) Because of this combined effect, pigmentation changes cannot be used as a criterion of measurement of differences between pullets in the component factors contributing to the egg record.

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RATE AND DATE OF SEEDING KANRED WINTER WHEAT AND THE RELATION OF SEEDING DATE TO DRY-LAND FOOT ROT AT AKRON, COLO.[1]

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INTRODUCTION

Experiments to ascertain the best rate and date to seed winter wheat have been conducted in the Great Plains area for various periods since the establishment of experiment stations in that area. Martin[2] summarized the results of investigations conducted in the Great Plains and Great Basin to 1925 as follows:

> The optimum rate of seeding for wheat is practically independent of soil type, moisture, locality, date of seeding, cultural treatment, and variety. The optimum date of seeding for winter wheat is independent of soil type, annual precipitation, variety, and rate of seeding, but is related somewhat to temperature, even though the same temperature conditions do not apply in all sections. Rates of 4 to 6 pecks per acre in general have produced the highest net yields of winter and spring wheats. Medium seeding of winter wheat usually is most favorable.

The results of two experiments, one started in 1920 to ascertain the best rate and date of seeding Kanred (C. I. 5146) winter wheat (*Triticum aestivum* L.) and a second one started in 1930 by H. Fellows to study the occurrence and severity of dry-land foot rot as affected by the date of seeding, will be discussed in this paper. The studies were conducted under dry-land conditions at the United States Dry Land Field Station, Akron,[3] Colo., where the average annual rainfall is approximately 17 inches.

EXPERIMENTAL METHODS

The soil of the Akron station is classified as Weld silt loam. It is a light brown loam underlaid at a depth of 12 to 15 inches by a calcareous layer. The subsoil is slightly heavier in texture than the surface soil.

In the studies of date and rate of seeding, plots were seeded on or near the following dates during the period from 1920 to 1937: August 15, September 1, September 15, October 1, and October 15. In 1938

and 1939, plantings were made weekly between August 15 and October 1. The wheat was seeded at the following per-acre rate on each date: 1 peck, 2 pecks, 3 pecks, 4 pecks, and 5 pecks, except in 1926, 1927, and 1928, when the 6-peck rate was used instead of the 1-peck rate. Four replications of each rate on each date were sown, two on fallow and two on cornland

From 1920 to 1925 all plantings were made with an 8-inch disk drill. After 1925 a 12-inch furrow drill was used. From 1920 to 1925 the plots were one-forty-fifth of an acre in size. After 1925 smaller plots, one-fifty-fifth of an acre, were used.

Dry-land foot rot occurs throughout the semiarid region of the United States. Its presence is noticeable in the early planted fields of winter wheat. The appearance of a moderately diseased field

FIGURE 1.—Winter wheat showing normal plants (*A*) and plants infected with dry-land foot rot (*B*).

resembles that of a field that has been subjected to extreme drought. The plants ripen prematurely and are more or less stunted. The wheat in the diseased portion of a field when viewed from a distance is more or less ashy gray in color. In a badly diseased field the plants may die before heading occurs.

The study of the relationship of dry-land foot rot to date of seeding was made on summer fallow. An additional set of plots seeded at 3 pecks per acre was planted at each date in the rate-and-date study.

In studying the amount and severity of foot rot, plants from 5 feet of row taken at random were pulled for each date of planting at or shortly before ripening and the crowns were examined for the presence and severity of foot-rot lesions. The crowns were cut the long way (fig. 1). The severity was determined mainly by the

extent and darkness of the lesions, and in part by the progress of decay in the crowns.

Analysis of variance [4] was used in the interpretation of the data. Snedecor's [5] F value was used to determine the significant departures.

The homogeneity tests [6] of the yield data of years for fallow and cornland indicate that the data for the various years cannot be considered to have been drawn from the same general population. Thus, the high value of χ^2 indicates that a valid determination cannot be made from the generalized standard error of the odds that one category is either greater or less than another, since the generalized standard error may be too small for the category with the greater dispersion and too large for the one with the lesser dispersion. It would seem valid, however, to use not only the generalized standard error but also the various interactions to estimate the random sampling fluctuations about the general mean, since only the dispersion about the general mean is considered and not the differences between means of specific categories. Therefore, in this paper F values were used to determine trends and not differences between specific rates and dates.

In a study of this kind several factors may influence the final results. Some of these, such as extremely low temperatures and moisture, may destroy part or all of the experiment. In analyzing the data an effort was made to discover some of the causes affecting the yield of winter wheat planted at different rates and dates. In order to do this and to eliminate the effect of as many different factors as possible, yields which were definitely affected by winter-killing, date of emergence, and lack of moisture have not been included in some of the analyses.

For the foregoing reasons only data from years in which the crops survived the winter and in which moisture was sufficient to produce a crop have been used. The data for the years 1925 to 1928, inclusive, 1934, 1935, 1938, and 1939 were not used for the following reasons: In 1925 part of the plots were destroyed by army cutworms and no yields were obtained in 1926, 1927, and 1928 the 1-peck rate was not included in the test; in 1934 the crop on cornland was a failure; in 1935 poor stands were obtained and, regardless of date of planting, the wheat emerged at the same time in the spring; and in 1938 and 1939 different dates of planting were used.

EXPERIMENTAL RESULTS

The results were analyzed separately for fallow and cornland. Data for 13 years (table 1) were used in the statistical analysis of the plots grown on summer fallow and for 12 years for the plots grown on cornland. The average annual yields in bushels per acre on summer fallow and cornland are given in table 2. The data for the years shown in table 2 are representative of those for the entire 18 years of the experiment.

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TABLE 1.—*Average yield per acre of Kanred winter wheat sown on summer fallow at various rates on different dates, for 13 of the years between 1920 and 1937*

Seeding rate per acre (pecks)	Yields per acre for wheat sown—					
	Aug. 15	Sept. 1	Sept. 15	Oct. 1	Oct. 15	Average
	Bushels	*Bushels*	*Bushels*	*Bushels*	*Bushels*	*Bushels*
1	10 2	14 5	15. 3	10. 6	9 9	12. 1
2	12 0	16. 2	15 5	11. 5	11. 1	13. 3
3	13. 0	16. 1	15. 4	12. 2	11. 3	13 6
4	13 8	16. 1	14. 9	12. 4	11. 8	13. 8
5	14. 5	16. 4	14. 5	12. 4	12 3	14. 0
Average	12 7	15. 9	15. 1	11. 8	11. 3	13. 4

TABLE 2.—*Yields per acre of Kanred wheat in rate- and date-of-seeding experiments on fallow and on cornland for certain years between 1920 and 1939*

FALLOW

Seeding dates	Seed-ing rate per acre	Yields per acre for wheat sown in —																		Aver-age yield per acre for all years	Aver-age num-ber of years used in statis-tical analy-sis
		1920	1921	1922	1923	1924	1926	1927	1928	1929	1930	1931	1932	1933	1934	1936	1937	1938	1939		
	Pecks	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Bu.	Years
Aug. 15–18	1	16.6	22.7	10.0	2.6	0		19.0	24.1	13.3	12.2	4.2	14.5	4.4	.4	12.2	20.4	1.2	.9	9.04	10.27
	2	27.9	25.1	10.5	5.5	0	2.9	17.3	22.1	14.8	18.4	2.7	16.3	4.4	.0	13.5	14.2	2.2	.4	11.41	12.05
	3	36.1	24.8	13.9	8.0	.0	.4	17.3	16.8	13.7	18.6	1.4	16.7	4.8	.0	14.5	14.0	4.8	1.2	11.93	13.00
	4	39.2	27.1	19.6	8.9	.0	.4	15.9	16.5	13.1	22.3	1.0	16.5	3.3	.0	14.0	12.8	6.6	1.6	12.31	13.77
	5	42.2	26.6	23.5	10.2	.0	.4	10.8	18.4	15.1	22.5	1.0	19.3	2.9	.0	14.7	10.5	7.1	1.4	12.97	14.50
Average		32.4	25.3	16.7	7.0	.0	.9	16.1	19.6	14.2	18.8	2.0	16.6	3.9	.0	13.7	14.4	4.2	1.1	11.7	12.7
Sept. 1–4	1	39.5	10.1	19.1	9.6	3.5	.2	21.1	40.8	14.4	25.2	3.1	20.4	1.9	.9	18.3	23.2	7.3	4.4	13.39	14.55
	2	43.9	13.7	19.8	12.2	6.1	.2	18.4	41.5	14.7	24.1	3.4	23.3	1.4	1.5	19.5	26.5	9.5	3.4	15.84	16.16
	3	40.3	15.2	21.6	12.0	6.6	1.0	12.3	43.4	14.4	24.2	3.4	23.8	.9	1.1	19.3	27.0	11.6	2.8	15.78	16.12
	4	41.8	17.4	21.4	14.5	8.2	1.0	10.5	42.7	13.4	23.2	3.5	21.5	.9	.8	18.1	23.9	12.4	2.3	15.61	16.12
	5	43.8	15.7	23.3	15.4	9.6	.8	9.6	42.2	13.4	19.8	5.6	22.9	.9	.7	17.7	24.3	13.7	1.8	15.71	16.39
Average		41.9	14.4	21.1	12.7	6.8	.6	14.4	42.1	14.2	23.3	3.8	22.4	1.2	1.0	18.6	25.0	10.9	2.9	15.3	15.9
Sept. 15–18	1	26.3	13.7	18.3	13.3	10.7	.8	17.1	46.3	13.7	21.1	7.2	22.8	2.2	5.3	17.9	26.2	20.2	6.9	15.05	15.28
	2	28.8	15.6	17.0	14.4	9.3	1.3	13.8	51.0	13.7	19.8	7.0	25.2	2.2	4.3	19.4	24.9	21.5	10.8	16.56	15.51
	3	27.9	16.5	20.4	14.1	9.8	.8	12.5	53.5	13.2	18.6	6.0	23.8	2.3	2.8	19.0	25.7	20.5	10.3	16.55	15.46
	4	28.5	15.2	19.7	12.9	9.8	.8	11.5	41.5	12.3	21.1	7.6	18.7	2.6	1.9	19.8	25.1	17.5	7.6	15.89	14.94
	5	23.4	15.6	18.7	13.9	8.0	.9	10.8	48.7	12.6	21.1	8.2	16.4	2.8	2.6	19.4	25.3	23.4	6.6	15.14	14.54
Average		27.0	15.3	18.9	13.7	9.5		13.1	48.2	13.1	20.3	7.4	21.3	2.4	3.3	19.1	25.4	20.6	8.4	15.8	15.1

Footnotes at end of table.

TABLE 2.—*Yields per acre of Kanred wheat in rate- and date-of-seeding experiments on fallow and on cornland for certain years between 1920 and 1939*—Continued

FALLOW

Seeding dates	Seeding rate per acre (Pcks)	Yields per acre for wheat sown in— 1920	1921	1922	1923	1924	1926	1927	1928	1929	1930	1931	1932	1933	1934	1936	1937	1938	1939	Average yield per acre for all years (Bu.)	Average number of years used in statistical analysis (Years)
Oct. 1–4	1	3.9	9.7	12.6	11.0	7.6	1.3	19.6	26.5	11.7	17.5	8.3	15.3		5.8	10.1	19.5	16.2	3.0	10.45	10.58
	2	4.2	11.9	14.7	12.1	6.8	1.1	20.0	40.6	11.6	20.0	6.8	17.0	4.5	5.7	10.7	23.3	16.1	2.0	12.04	11.50
	3	6.7	15.5	16.7	12.8	7.6	1.3	16.7	37.5	11.6	20.4	7.9	18.6	4.4	6.0	10.7	21.2	15.3		13.47	12.25
	4	8.3	16.5	16.6	12.8	6.3	1.7	16.9	46.3	11.6	20.4	7.5	18.1	3.8	5.7	12.0	20.9	16.5	5.2	13.66	12.38
	5	6.1	17.1	18.0	13.8	1.0	.6	18.3	46.1	13.2	22.3	7.5		4.5	4.2	11.1	19.0			14.31	12.45
	6																				
Average		5.9	14.2	15.8	12.5	7.0	1.3	18.3	39.4	11.8	20.1	7.6	17.4	4.4	5.4	10.9	20.8	16.4	3.4	12.8	11.8
Oct. 15–18	1	5.4	14.0	14.1	11.9	5.1	.8	23.3	30.8	11.8	13.6	8.1	14.1	3.7	4.5	6.4	14.0			9.82	9.82
	2	6.8	16.1	17.2	13.9	3.9	1.0	19.6	31.6	13.4	16.1	9.5	15.6	4.1	5.7	7.3	16.3			12.36	10.98
	3	7.8	17.3	17.2	13.7	5.3	1.0	21.7	37.6	12.0	15.3	7.9	15.4	3.9	5.1	7.7	16.1			12.34	11.18
	4	12.5	18.5	18.6	15.0	3.8	1.3	22.7	32.6	11.6	15.6	7.3	14.0	3.4	4.6	7.7	19.5			13.24	11.70
	5	12.8	17.4	19.4	14.3	4.4	.2	19.6	32.3	11.8	16.5	7.5	14.8	4.5	5.0	9.0	20.9			13.41	12.18
	6																				
Average		9.1	16.7	17.2	13.3	4.5	.9	21.3	32.9	12.1	17.1	8.0	14.8	3.9	5.2	7.6	17.3	16.4		12.2	11.3

CORNLAND

Seeding dates	Seeding rate per acre (Pcks)	Yields per acre for wheat sown in— 1920	1921	1922	1923	1924	1926	1927	1928	1929	1930	1931	1932	1933	1934	1936	1937	1938	1939	Average yield per acre for all years (Bu.)	Average number of years used in statistical analysis (Years)
Aug. 15–18	1	7.6	10.1	5.0	6.8	.0	.8	30.0	17.3	7.1	16.6	10.6	7.6	.0		5.8	11.0	3.5	.4	6.58	7.35
	2	10.6	10.9	5.8	10.1	.0	.8	21.3	16.9	7.8	26.3	10.3	5.2	.0		6.3	12.9	5.6	.4	9.61	9.10
	3	16.4	13.7	9.3	11.8	.0	.8	17.1	11.7	8.9	26.7	9.2	9.0	.0		6.9	13.6	5.4	.6	10.00	10.43
	4	19.4	13.8	13.0	11.8	1.0	1.0	16.0	11.5	8.9	27.5	9.0	10.0	.0		5.9	12.6	6.0	.4	10.99	10.99
	5	23.2	14.1	15.2	13.3	.1	.8	7.9	10.1	10.1	26.5	9.1	9.6			5.8	12.2	6.7		10.92	11.59
	6																				
Average		15.4	12.5	9.7	10.7		.8	17.3	13.5	8.5	24.7	9.6	8.9	.0		6.1	12.4	5.4	.4	9.2	9.9

Sept. 1-4

Average

Sept. 15-18

Average

Oct. 1-4

Average

Oct. 15-18

Average

RATE AND DATE OF SEEDING KANRED WINTER WHEAT
NO FALLOW

The analysis of variance for the plots grown on summer fallow is given in table 3. The F values indicate that there are significant differences in yield between rates and between dates of seeding, and that rates within dates tend to hold the same relative positions. Rates within years also show a tendency to remain constant. However, dates within years vary, and the same date may not give the highest yield every year.

TABLE 3.— *Analysis of variance for rates and dates of seeding Kanred winter wheat on summer fallow for 13 of the years between 1920 and 1937*

Variation due to—	Degrees of freedom	Sum of squares	Variance	Values of F, using for the divisor [1]—			
				Error	Rates × dates	Interaction with year	Rates
Years	12	29,356.893	2,446.408	229.688**			
Blocks	13	2,118.821	162.986	15.302**			
Rates	4	296.038	74.010	6.949**	5.567**	5.234**	
Dates	4	2,139.418	534.854	50.216**	40.233**	2.079	7.227
Rates×dates	16	212.701	13.294	1.248			
Rates×years	48	678.752	14.141	1.328			
Dates×years	48	12,351.632	257.326	24.160**			
Rates×dates×years	192	1,293.943	6.739				
Error	312	3,323.036	10.651				
Total	649	51,771.237					

[1] * Indicates significance at 5-percent level; ** at 1-percent level.

Using rates×years or dates×years as error, there is still a significant difference for rates of planting, but because of the variability within different years the different dates are not significant.

When the year effect is eliminated and dates×rates is used as error, there is a significant difference for both rates and dates. This indicates that on the average certain rates and certain dates give better yields, and that other rates and dates give poorer yields.

Dividing rates by dates, an F value of 7.227 was obtained, which is greater than the 5-percent point and indicates that dates are more important than rates.

The results are presented graphically in figure 2. This figure and table 1 indicate that the 1-peck rate gives the lowest yield, and that any increase in yield when seeding is at a higher rate than 2 pecks is slight and not significant. The September 1 and 15 dates give the highest yields, with a marked drop before and after these dates. From these results it may be concluded that when winter wheat is planted at the proper time, between September 1 and 15, 2 pecks is the best rate of seeding.

RATE AND DATE OF SEEDING KANRED WINTER WHEAT ON CORNLAND

In the studies on cornland an additional year, 1924, has been left out since no stands were obtained. The average yields were somewhat lower for wheat following corn than for wheat following fallow.

The average yields are given in table 4, and the variance analysis is presented in table 5.
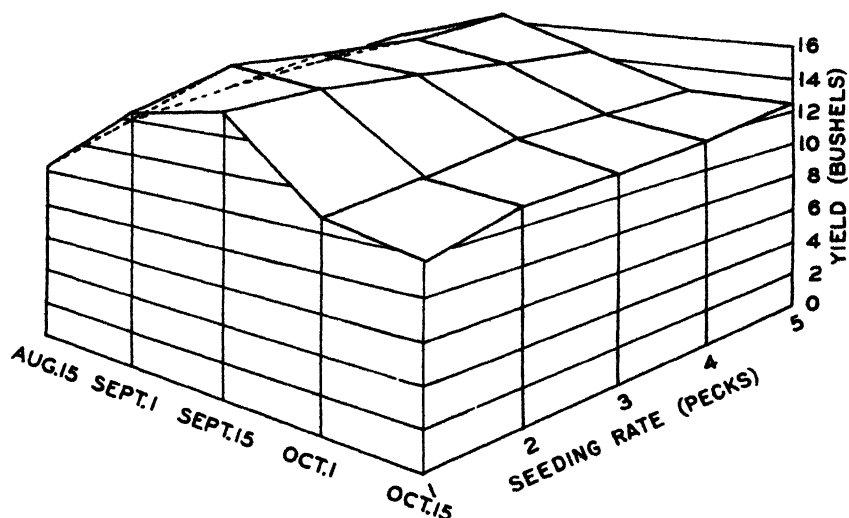
FIGURE 2.—Seeding rates, dates of sowing, and yields of Kanred winter wheat seeded on fallow.

TABLE 4.— *Average yields per acre of Kanred winter wheat sown on cornland at various rates on different dates for 12 years between 1920 and 1937*

Seeding rate per acre (pecks)	Yields per acre for wheat sown—					
	Aug. 15	Sept. 1	Sept. 15	Oct. 1	Oct. 15	Average
1	7 3	11 2	11 4	6. 7	5 9	8. 5
2	9 1	11. 6	11 3	6. 7	6 7	9 1
3	10. 4	12 1	10 6	6. 9	6. 9	9 4
4	11 0	12 3	10 7	6 8	7. 1	9 5
5	11 6	12 3	10 2	7 6	7 7	9 9
Average	9 9	11 9	10 8	7. 0	6 9	9. 3

TABLE 5.— *Analysis of variance for rates and dates of seeding Kanred winter wheat on cornland for 12 of the years between 1920 and 1937*

Variation due to—	Degrees of free-dom	Sum of squares	Variance	Values of F, using for the divisor[1] —			
				Error	Rates × dates	Interaction with year	Rates
Years	11	21, 596. 610	1, 963. 328	212 596**			
Blocks	12	5, 214. 775	434. 565	47. 056**			
Rates	4	125. 158	31. 290	3 388**	1. 993	2 159	
Dates	4	2, 493 107	623 277	67. 491**	39 602**	3 539*	19. 919**
Rates×dates	16	251 243	15 703	1 700			
Rates×years	44	637 552	14 490	1 569*			
Dates×years	44	7, 748 353	176 099	19 009**			
Rates×dates×years	176	881 176	5 007				
Error	288	2, 659 625	9 235				
Total	599	41, 607. 599					

[1] * indicates significance at 5-percent level. ** at 1-percent level

The *F* value in table 5 shows that both rates and dates differ significantly. The *F* value for rates×dates indicates that the rates are fairly constant within dates. Both rates and dates vary within different years.

When the dates×year interaction is used as error, we find a significant *F* value for dates, indicating that there is a significant difference in yield due to dates of planting. However, when the rates× years interaction is used as error, the *F* value is not significant, indicating that yields due to rates of planting do not differ significantly. However, when the variability due to years is eliminated and the rates×dates interaction is used as error, there is a highly significant *F* value for dates and a low and not significant value for rates. As in the case of fallow, dates are again more important in planting winter wheat, as indicated by the *F* value of 19.919, which is greater than the 1-percent point.

The data in table 4 and the graphical representation of the data in figure 3 show a slight increase in yield on the average for seedings
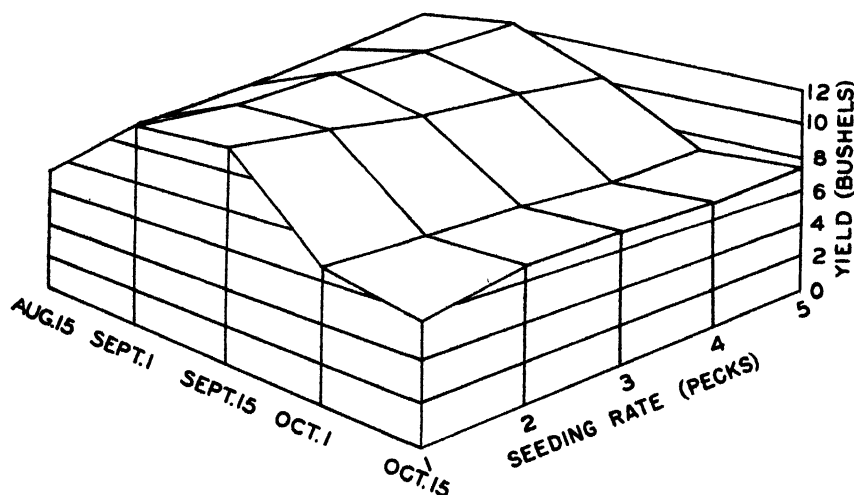
FIGURE 3.—Seeding rates, dates of sowing, and yields of Kanred winter wheat seeded on cornland.

above the 1-peck rate but quite a variation within dates. The data for date of planting show that planting on September 1 gave the highest average yield. This holds true throughout the range of rates, with the exception of the 1-peck rate, which is slightly higher on September 15. The early dates reverse their position for the 4- and 5-peck rates. However, basing results on average yield, it would seem safe to recommend that not to exceed 2 pecks of seed should be used and that the period from September 1 to 15 is the best time to plant winter wheat on cornland.

RELATION OF DRY-LAND FOOT ROT TO DATE OF PLANTING

Estimates of the amount and severity of infection with dry-land foot rot are given in table 6. Yields from adjacent plots seeded at the same rate in the rate-and-date studies on fallow are given in the

last column of the table. In all years infection and severity were greatest for the early seedings and descended progressively with the date of seeding. In studying the relationship between disease and yield, correlation coefficients were ascertained. In order to overcome the variability between different years, the yield for each date was calculated in percentage of the highest yield for that year. The highest yield for each year was considered as 100 percent, and the yields for the first three dates of planting were expressed as percentages of this value. By this means correlation coefficients of

TABLE 6.—*The relation of date of seeding Kanred winter wheat to development of dry-land foot rot, 1930–38, and yields from adjacent plots in the rate-and-date studies on fallow, seeded at approximately the same dates*

| Dates | | Dry-land foot rot readings on crowns | | Yield per acre |
Planted	Emerged	Plants diseased	Plant severity	
1930-31	*1930-31*	*Percent*	*Percent*	*Bushels*
Aug 18	Aug. 24	100 0	100 0	1 4
Sept 1	Sept 8	94 0	80 0	3 3
Sept 15	Sept 23	92 0	10 0	6 9
Oct. 1	Oct. 10	86 0	2 0	7 9
Oct 15	Nov. 10	46 0	Trace	7 9
1931-32	*1931-32*			
Aug 15	Aug. 22	68 5	10.0	16 7
Sept. 1	Sept. 8	53 3	30.0	23 8
Sept 15	Sept 24	40 8	25 0	23.8
Oct 1	Oct 15	30 0	5 0	18 3
Oct 15	Apr. 5	25.7	1.0	15 4
1932-33	*1932-33*			
Aug. 15	Aug. 22	90 0	25.0	4 8
Sept. 1	Sept 8	94 0	20 0	9
Sept. 15	Sept. 24	22 0	.5	2.3
Oct. 1	Oct 11	.0	.0	4 4
Oct 15	(¹)	.0	.0	3 9
1933-34	*1933-34*			
Aug. 15	Aug. 28	97 6	75 0	0
Sept. 1	Sept 7	88 8	60 0	1 1
Sept. 15	Sept. 21	97.2	50 0	2.8
Oct 1	Oct 10	52 9	20 0	6 0
Oct. 15	Oct 30	6 5	5.0	5 7
1934-35	*1934-35*			
Aug. 15	Aug. 21	48 8	12 1	0
Sept. 4	Sept. 11	57.1	10 6	.0
1935-36	*1935-36*			
Aug. 15	Aug 20	85 9	41.0	14. 5
Sept. 1	Sept. 6	91 5	53.0	19 3
Sept. 15	Sept. 23	52 8	12.0	19 0
Oct. 1	Oct. 9	0	0	10 7
1936-37	*1936-37*			
Aug. 15	Aug 20	95 7	50 5	14.0
Sept. 1	Sept. 9	84.6	32 5	27 0
Sept. 15	Sept. 23	51 4	17 9	25 7
Oct. 1	Oct. 15	23.2	5.0	21 2
Oct. 15	Nov. 1	10 5	5 0	16 1
1937-38	*1937-38*			
Aug. 16	Aug 23	36.8	... .	4 8
Sept. 1	Sept. 6	44.1	. .	11. 6
Sept. 15	Sept. 21	6 5		20. 5
Oct. 1	Oct. 9	.0	. ...	16. 1
1938-39	*1938-39*			
Aug. 15	Aug. 24	82 0	41 1	1. 2
Sept. 1	Sept 7	68.0	27 9	2 8
Sept. 15	Sept. 23	47.0	6 7	10 3
Oct. 1	Oct. 14	.0	.0	5 2

¹ Early winter.

−0.34 and −0.33 were found between yield in percentage of the highest yield and percentage of foot rot and severity, respectively. Neither correlation can be considered significant. The later dates of planting were not considered, since foot rot did not seem to be a factor in affecting the yield of October 1 and October 15 plantings. Table 6 shows that the percentage severity approaches zero for the October 1 and 15 plantings.

Since percentage infection and severity act together in the total damage done to the plant, a correlation between percentage infection times percentage severity and the percentage yield for the first three dates of planting based on the highest yield in each year was ascertained, and a correlation coefficient of −0.5588 with a p value of <0.01 was obtained (table 7), indicating a decrease in yield with an increase in disease. There was a decrease in yield after October 1, but this decrease was not caused by disease, as indicated by the data in table 6.

TABLE 7.— *Percentage of plants diseased × severity of infection, and yield of Kanred winter wheat in percentage of yield for the best date at the 3 peck rate of seeding for each year for 7 of the years between 1930 and 1938*

PERCENTAGE DISEASED × SEVERITY

Date of seeding	1930-31	1931-32	1932-33	1933-34	1935-36	1936-37	1938-39
Aug. 15 ...	100	7	22	73	35	48	34
Sept. 1	75	16	19	53	48	27	19
Sept. 15.	9	10	1	49	6	9	3

YIELD IN PERCENTAGE

	1930-31	1931-32	1932-33	1933-34	1935-36	1936-37	1938-39
Aug 15 ...	18	70	100	0	75	52	12
Sept 1.	42	100	19	18	100	100	27
Sept 15.	87	100	48	47	98	95	100

YIELD-TEMPERATURE RELATIONSHIPS

To find whether there is a critical temperature for planting winter wheat in order to obtain the highest yield, the relationship between the average "mean daily" temperature for the week before planting and the average yield in percentage of the highest yearly yield was ascertained for the August 15 and September 1 plantings.

In making these calculations the data from 1920 and 1930 were not used because of delayed emergence of wheat planted on the first two dates. The data from the years 1924, 1931, and 1933 were not included since the yields were all low because of lack of moisture. Table 8 gives the yields of Kanred wheat in percentage of the highest yearly yield calculated from the average of all rates within each date for each year and the average mean daily temperature for the week before planting. A correlation coefficient of −0.0898 was obtained. For the years studied this does not indicate any relationship between average mean daily temperature the week previous to planting on August 15 and September 1 and yield of Kanred winter wheat.

TABLE 8.—*Yield of Kanred wheat sown August 15 and September 1, expressed as the percentage of the highest yearly yield, and the average mean daily temperature the week preceding planting for 7 of the years between 1921 and 1937*

YIELD

Date	1920–21	1921–22	1922–23	1928–29	1931–32	1935–36	1936–37
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Aug. 15	100	79	51	100	74	72	57
Sept. 1	57	100	93	100	100	97	98

AVERAGE MEAN DAILY TEMPERATURE 1 WEEK PRECEDING PLANTING

	°F.	°F.	°F.	°F.	°F.	°F.	°F.
Aug. 8–15	65.9	71.4	74.6	73.9	70.9	77.6	78.5
Aug 24–31	63.9	75.1	76.2	74.8	69.0	68.2	72.8
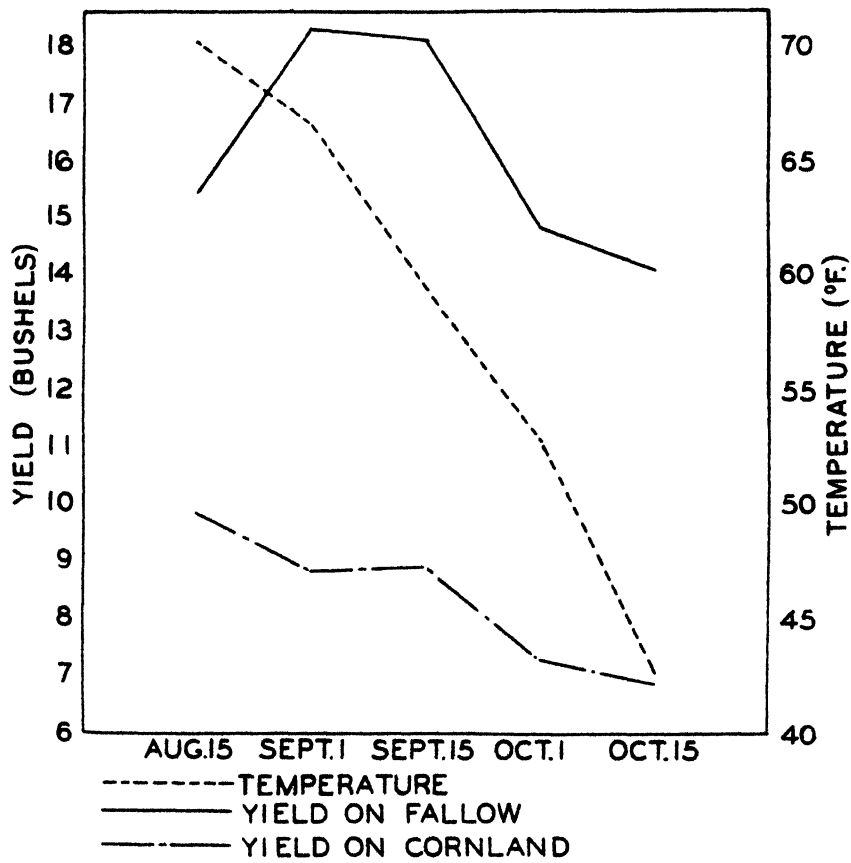
FIGURE 4.—Yield of Kanred winter wheat planted on fallow and on cornland at different dates and the average "mean daily" temperature for the first 3 weeks after planting.

Since the start of growth of wheat in the fall depends somewhat upon moisture and temperature after the wheat is planted, a further study was made in the years in which moisture was sufficient to

produce average yields. This was done to ascertain the influence of temperature for the first 3-week period after planting. The average mean daily temperature for this period was correlated with the yield in percentage of the highest yearly yield of winter wheat grown on fallow. From the data previously described, it is evident that there is an increase in yield up to September 1 and a sharp reduction in yield after September 15. In order to ascertain the effect of temperature on yield, the data from the different dates were divided into two periods: (1) August 15 to September 1, and (2) September 1 to October 15. Table 9 presents the yield data and the average mean daily temperatures for 7 of the years between 1921 and 1937. A significant negative correlation coefficient of −0.5498 was obtained for the yield and temperature relationship for the first two dates, and a significant positive correlation of +0.5069 was found for the four dates from September 1 to October 15. Figure 4 presents the data for the temperature and yield relationships on fallow and on cornland. The yield curve for the winter wheat planted on cornland drops off gradually throughout and does not show the negative effect of temperature for the early periods. This change is due to high yields for the August 15 planting in 1921 and 1937. When the entire period studied is considered, the data follow the same trend as that for wheat on fallow.

TABLE 9.—*Yield of Kanred winter wheat sown at different dates, expressed as the percentage of the highest yearly average yield for all rates of sowing, and the average mean daily temperatures for 3 weeks after each planting for 7 of the years between 1921 and 1937*

YIELD

Dates	1920–21	1921–22	1922–23	1928–29	1931–32	1935–36	1936–37
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Aug. 15	100	79	51	100	74	72	57
Sept. 1	57	100	93	100	100	97	98
Sept. 15	60	90	100	92	95	100	100
Oct. 1	56	75	91	83	78	57	82
Oct. 15	66	82	97	85	66	40	68

AVERAGE MEAN DAILY TEMPERATURE 3 WEEKS AFTER PLANTING

	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*	° *F.*
Aug. 16–Sept 7	63.6	70.4	74.9	70.1	70.7	68.0	73.0
Sept. 1–22	64.7	64.4	67.3	66.2	69.6	65.2	68.4
Sept. 16–Oct. 7	60.4	61.2	62.7	57.6	60.5	59.0	54.5
Oct. 1–22	54.2	56.7	51.7	51.6	54.2	52.1	48.7
Oct. 16–Nov. 7	40.7	49.9	45.0	39.0	49.6	37.9	37.0

In order to ascertain the best possible average mean daily temperature for the 3 weeks after planting, regressions were calculated for the two correlation coefficients. From this information lines were drawn (fig. 5), and the point of intersection of the two regression lines was found to be 65.8° F. These data indicate that under Colorado conditions the highest yield of winter wheat is most likely to be obtained when plantings are made on a date preceding a 3-week period in which the average daily mean temperature is 65.8° F. This temperature condition has occurred most often between September 1 and 15 during the years of the study. Other factors such as moisture and winter-killing may alter the results expected.

DISEASE-TEMPERATURE RELATIONSHIPS

Since a significant correlation coefficient was obtained for yield and temperature for a 3-week period after planting, and for yield in the early dates and disease infection × severity, it follows that there should be a relationship between temperature for the 3-week period after planting and disease infection × severity. Since the disease study included only data from 7 years between 1930 and 1938, the data for these 7 years of the study were used. Table 10 and figure 6
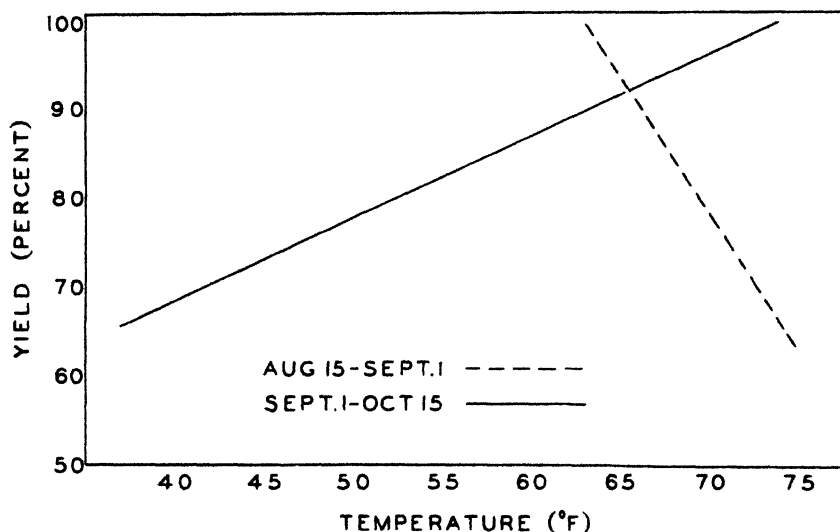
FIGURE 5.—Regression lines showing the most favorable average mean daily temperature for the 3 weeks after planting Kanred winter wheat. The point of intersection of the two lines is considered as the best possible estimate.

TABLE 10.—*Percentage of plants diseased × severity of infection, and the average mean daily temperature for 3 weeks after planting Kanred winter wheat for 7 of the years between 1931 and 1939*

PERCENTAGE DISEASED × SEVERITY

Dates	Years grown						
	1930–31	1931-32	1932–33	1933–34	1935–36	1936–37	1938–39
Aug. 15	100	7	22	73	35	48	34
Sept 1	75	16	19	53	48	27	19
Sept 15	9	10	1	49	6	9	3
Oct 1	2	2	0	11	0	1	
Oct. 15	0	0	0	0		1	

AVERAGE MEAN DAILY TEMPERATURE 3 WEEKS AFTER PLANTING

	° F.	° F.	° F.	° F.	° F	° F.	°F
Aug. 16–Sept. 7	68.0	70.7	70.4	69.8	68.0	73.0	69.3
Sept. 1–Sept. 22	64.5	69.6	64.9	68.5	65.2	68.4	62.6
Sept. 16–Oct. 7	58.5	60.5	57.1	61.2	59.0	54.5	64.4
Oct. 1–Oct. 22	49.0	54.2	49.3	53.1	52.1	48.7	
Oct. 16–Nov. 7	41.6	49.6	41.7	46.4	37.9	37.0	

present the data. A positive correlation of +0.5919 with a p value of 0.01 was found for disease infection $\times$ severity and the average mean daily temperature for a 3-week period after planting for the following dates of planting: August 15, September 1, September 15, October 1, and October 15. This indicates a direct relationship between disease and temperature.

In order to find the type of regression curve which would best fit the data, both linear and quadratic regressions were calculated and the better fit ascertained by variance analysis (table 11).
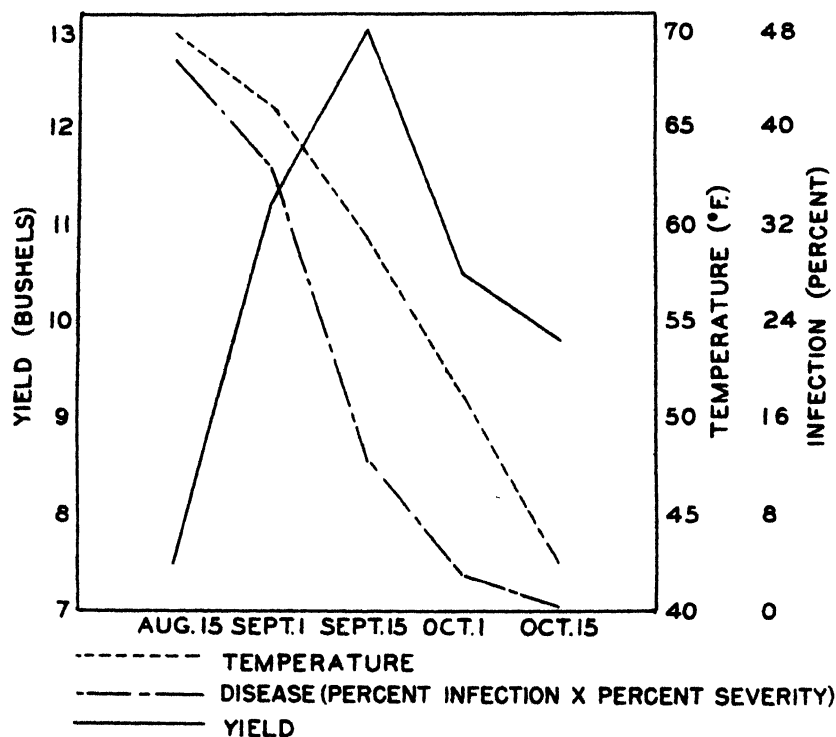
FIGURE 6.—Relationship of average mean daily temperature, percentage of infection $\times$ disease severity and yield of Kanred winter wheat planted at different dates in the fall.

DISCUSSION

There has long been a belief that temperature at the time of and after planting winter wheat effects the yield. The influence of temperature after planting on the prevalence of bunt in winter wheat when the seed has been inoculated with bunt spores is well known. Temperature after planting may influence the incidence and severity of dry-land foot rot in areas where it is an important factor in reducing yields, as indicated by the negative correlation coefficient of -0.5588, $p < 0.01$, between infection in percent $\times$ severity in percent and yield in percent for the early planting dates, and the significant negative correlation between yield in percent and average daily mean tempera-

ture for a 3-week period after planting (table 9) for the early planting dates, August 15 and September 1.

The foregoing data, together with the positive correlation coefficient of $+0.5919$, $p < 0.01$, between disease infection $\times$ severity and the average mean daily temperature for a 3-week period after planting, clearly indicate that temperature affects yield and that part of the reduction in yield in the early plantings is due to the effect of temperature on the incidence of dry-land foot rot. After September 15 the effect of foot-rot infection is reduced, and low temperature evidently is the main factor in reducing yields. This is indicated by the correlation coefficient of $+0.5069$, $p < 0.01$, for the average mean daily temperature for the 3-week period after planting for the four dates of September 1, September 15, October 1, and October 15, and yield.

TABLE 11.—*Variance analysis comparing linear and quadratic regression of the percentage of disease* $\times$ *severity and temperature for the 3-week period after planting*

Variance due to—	Degrees of freedom	Sum of squares	Mean square	F
Quadratic—linear regression	1	11,008.45	11,008.45	224.8**
Deviation from quadratic regression	24	1,174.71	48.95	
Deviation from linear regression	25	12,183.16		

¹ **Greater than the 1-percent point   The analysis indicates that quadratic regression fits the data better than linear regression.

Moisture or lack of moisture, as previously shown, may be an important factor in influencing the growth and final yield of the winter wheat crop planted at different dates. In the early-planted crops where a heavy growth occurs in the fall, followed by a dry winter and spring, the plants may exhaust the soil moisture, and loss of stand and poor yields may result. With later dates of planting, this effect is reduced since time for growth in the fall is reduced. However, the fact that disease severity was greatest in the early plantings and that it was correlated negatively with yield indicates that disease was also an important factor in reducing yields.

The later-sown plots, while using less moisture, were retarded in growth, and temperature evidently had an important effect on this retardation, as indicated by the significant positive correlation between yield and temparature for the 3 weeks after planting. Wheat is known to go into a dormant period when the temperature drops below a certain point. The amount of growth made by the plant before this period would determine its ability to develop after growth started in the spring. The better-developed plants would have an advantage in the spring over those less well developed, and under favorable conditions would grow into larger and healthier plants.

SUMMARY

The data from two studies presdnted in the foregoing paper indicate:

That planting winter wheat at different dates results in significant differences in yields on summer-fallow in eastern Colorado.

Winter wheat should be planted between September 1 and 15 to obtain the best yields on either summer-fallow or cornland.

One peck of seed per acre is not sufficient for all conditions but 2 pecks are sufficient for both fallow and cornland. Three pecks or more do not increase yields enough to justify the use of the additional seed.

From studies made to discover the cause of the reduction in yield arising from the different dates of planting, the following conclusions are drawn:

The effect of high temperature on the incidence of dry-land foot rot and the resulting effect of the disease on the plant is one of the main factors in reducing yields in the earlier plantings (August 15).

Low temperature is the main factor in reducing yields in plantings made after September 15.

Other factors such as moisture and winter-killing may act independently of rates or dates of planting in reducing yields.

TOXICITY AND ACCUMULATION OF CHLORIDE AND SULFATE SALTS IN PLANTS [1]

By FRANK M. EATON [2][3]

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INTRODUCTION

The subject of salt toxicity, or "alkali," has been the object of experimental attention almost since the beginning of irrigation developments in the Western States. Recent advances in an understanding of the role of trace elements in plant growth and in the development of methods of physiological research have not only pointed to the need and provided the means for new studies of salt toxicity but they have also afforded an insight into some of the difficulties encountered by early investigators. Although salt toxicity and the quality of irrigation waters constitute but one aspect of the problem of profitable and permanent agriculture under irrigation, it is none the less a significant one, since decisions on the suitability of water supplies, the quantities of water required for soil leaching, and the financial advantages of drainage works and their capacities can be based intelligently only upon quantitative information on salt toxicity to plants. The wise selection of crops for saline soils must likewise rest on a knowledge of the relative tolerances of species and varieties of plants to the salt constituents of soil solutions.

When the present work was undertaken in 1934, there was a widespread belief in the western irrigated areas that if the neutral salts were below substantial concentrations the plants would be uninjured whereas if salts were above these concentrations injury would be pronounced. It appeared to the author, on the basis of evidence drawn from the literature as well as from observations in the field and in minor experiments, that there were weaknesses in this high-tolerance point of view and that additional investigations were needed. The experiments as originally undertaken had as a principal objective a study of the comparative reactions to chloride and sulfate salts of a number of crop plants grown together in a series of outdoor sand cultures differentially supplied with salts added to a base nutrient solution. Particular attention was paid to the development of plant symptoms that might be of diagnostic value under field conditions. The concentrations of salt constituents in the expressed sap of the plants were measured as a means of correlating injury with salt

accumulation. Following the outdoor experiment, a number of greenhouse experiments were conducted for the purpose of obtaining a clearer idea of the character of the salt-injury curves through a series of low as well as intermediate and high concentrations of chloride and sulfate. The results of the first-mentioned work have been briefly referred to in an earlier paper *(10)*.[4]

The literature bearing on the toxicity of chloride and sulfate salts, though not particularly extensive, is difficult to coordinate and summarize. The experimental procedures employed both in the field and in the laboratory have been diverse, and there is wide variability in the tolerance of different plants to chloride and sulfate ions. Moreover, the reactions of plants are in some measure dependent upon whether the chloride and sulfate are present as calcium, magnesium, or sodium salts, and upon climatic conditions as well. There has been no extensive work on this problem in recent years, and it has seemed better to refer in the text to results directly related to the present data rather than to undertake a general review of the literature.

EFFECT OF CHLORIDE AND SULFATE SALTS ON A SERIES OF CROP PLANTS IN OUTDOOR SAND CULTURES (EXPERIMENT 1)

CLIMATIC CONDITIONS

The summer temperatures at Riverside, Calif., which is 40 miles inland from the Pacific Ocean, are not so high (table 1) as those prevailing in many of the interior valleys of the Southwest, nor are they as low as those of the coastal area. The climate at Riverside, usually tempered by the prevailing westerly winds from the Pacific, is occasionally hot and dry as a consequence of north and easterly winds coming off the Mojave and Colorado Deserts. The noonday relative humidity customarily falls in the 30-to-50 percent range, and the nights are almost always cool.

METHODS AND MATERIALS

The six sand beds used in this experiment have previously been described *(9)*. The eight crops grown in short parallel rows in each

TABLE 1.—*Climatological data* [1] *for the period of the outdoor sand-culture experiment at Riverside, Calif., in 1934*

Month	Temperature						Relative humidity at 12 noon		
	Maximum			Minimum			Lowest	Highest	Mean of month
	Lowest	Highest	Mean of month	Lowest	Highest	Mean of month			
	°F.	°F.	°F.	°F.	°F	°F.	Percent	Percent	Percent
May	74	107	88.2	41	59	50.2	10	48	31.1
June	68	97	82	46	63	53.7	22	68	46.1
July	88	117	95.9	51	68	58.7	14	51	33.6
August	90	102	96.1	52	64	57.4	15	46	32.4
September	61	104	92.8	41	63	55.2	15	76	32
October	65	104	86	44	60	50	18	84	37.6
November	61	93	74	34	52	44	15	94	43.6

[1] Measurements made at Riverside by the University of California.

[4] Italic numbers in parentheses refer to Literature Cited, p. 397.

of the beds were, in order of planting from the north end to the south end, as follows: Dwarf milo (*Sorghum vulgare* Pers.), Acala cotton (*Gossypium hirsutum* L.), rooted lemon cuttings (*Citrus limonia* Osbeck), barley (*Hordeum vulgare* L.), navy beans (*Phaseolus vulgaris* L.), sugar beets (*Beta vulgaris* L.), alfalfa (*Medicago sativa* L.) (three cuttings), and tomatoes (*Lycopersicon esculentum* Mill.). All but the lemons were carried to maturity.

The treatments consisted of a control bed supplied with a base nutrient solution, which was the same in all the cultures, two chloride beds, one with 50 and one with 150 milliequivalents of chloride ion per liter, and three sulfate beds in which concentrations of 50, 150, and 250 milliequivalents, respectively, of sulfate ion per liter were maintained. Fifty percent of the chloride and sulfate ions was added as sodium salts, with the remaining 50 percent divided between calcium and magnesium salts. Equal proportions of the latter were used in the two chloride beds and also in the 50-sulfate bed, but because of the limitations of calcium sulfate solubility, magnesium sulfate was substituted for calcium sulfate above 20 milliequivalents per liter in the 150- and 250-sulfate beds. Tap water was used in the preparation and replenishment of the culture solutions.

The culture of a series of crop varieties together in sand beds or water cultures for a study of comparative physiology affords the best possible assurance that all are subjected to the same substrate conditions, including such factors as the concentrations of nutrient and toxic elements, temperatures in the root zone, oxygen, carbon dioxide, and water supply. Free exposure out of doors likewise tends to provide nearly the same gross climatic complex for all members of a planting. Nevertheless inequalities in exposure are created by reason of the differential responses of the several crops grown in each of the sand beds to superimposed variables, in this case the chloride and sulfate salts. Since plants are reduced in size by these salts, their exposure to light and wind is increased in relation to the control plants. This condition exists also in field plantings.

The average composition of the tap water and the concentration of the constituents added in the preparation of the nutrient solutions are shown in table 2. The tap water contained 0.15 part per million of boron, and 0.85 p. p. m. was added in the preparation of each of the new solutions; 0.5 p. p. m. of manganese was also added. Iron was added daily as 10 ml. of a 5-percent iron tartrate solution. Zinc was not added directly, but measurements made on solutions of a

TABLE 2.—*Composition of tap water and concentrations of salt constituents added to tap water in preparation of nutrient solutions*

Item	Constituent (milliequivalents per liter)								
	Ca	Mg	Na	K	HCO₃	SO₄	Cl	NO₃	H₂PO₄
Tap water	1.84	0 56	1 63	0 17	2.90	0 61	0.63	0 05	....
Constituents added.									
Control	6.00	2.0	....	8	....	2		13	1
50-chloride	18.5	14.5	25	8	....	2	50	13	1
150-chloride	43.5	39 5	75	8	....	2	150	13	1
50-sulfate	18.5	14.5	25	8	....	52	....	13	1
150-sulfate	26.0	57.0	75	8	....	152	....	13	1
250-sulfate	26.0	107 0	125	8	.	252	....	13	1

subsequent experiment indicated that sufficient quantities were derived from the tap water and from impurities in the salts used in making up the solutions.

The eight crops were planted in parallel 18-inch rows in each of the six sand beds on April 17, 1934. These plantings were carried beyond germination with the base nutrient solution plus one-fifth of the final quantity of the chloride and sulfate salts. Additional fifths of salt were added on April 30 and May 2, 4, and 6. A half portion of each of the solutions with the final concentration of salt was then made up, used to flush the sand, and discarded. On the following dates new solutions were substituted for those in use: May 12 and 30, June 13 and 28, July 12, August 2 and 24, September 6, and October 5. The nitrate concentrations in all solutions were measured periodically, and deficiencies from the initial concentrations were replaced by additions of potassium nitrate or ammonium nitrate on July 19, July 26, August 16, and September 20.

The 180 liters of solution applied to the surface of the sand at each flooding of the beds was sufficient to replace by about one and a quarter times the solution held against gravity by the sand. The culture solutions returning to the lower reservoir were brought to volume with tap water after each use and then drawn up by suction into the upper reservoir to be used again. The solution held by the sand was replaced once each morning while the plantings were small, but as they became larger the operation was repeated a second time shortly after noon.

TOLERANCE

The relative tolerances of the series of crops to chloride and sulfate salts, as observed under the conditions of this experiment, are reported in table 3.

TABLE 3.—*Growth and yield* [1] *of plants in experiment 1*

LEMONS, ROOTED EUREKA CUTTINGS (APR. 17 NOV. 19)

Nutrient solution	Plants surviving	Leaves retained		Total (leaves, stems, and roots)	
		Dry weight	Relative dry weight	Dry weight	Relative dry weight
	Number	*Grams*	*Percent*	*Grams*	*Percent*
Control	3	26	100	46	10
50-chloride	3	7	27	13	2
150-chloride	0	0	---	---	---
50-sulfate	3	21	81	39	85
150-sulfate	3	8 7	32	15 2	33
250-sulfate	2	2. 6	10	7. 1	15

BEANS, NAVY (APR. 17–JULY 13)

Nutrient solution	Plants surviving	Seed		Entire plants	
		Dry weight	Relative dry weight	Dry weight	Relative dry weight
	Number	*Grams*	*Percent*	*Grams*	*Percent*
Control	5	347	100	671	100
50-chloride	5	135	39	271	40
150-chloride	0				
50-sulfate	5	225	65	443	66
150-sulfate	5	89	25	160	24
250-sulfate	0	0			

[1] Sums of weights of plants in 18-inch rows are represented, unless otherwise indicated.

TABLE 3.—*Growth and yield of plants in experiment 1*-- Continued

MILO, DWARF (APR. 17-SEPT. 19)

Nutrient solution	Plants surviving	Grain		Entire plants	
		Dry weight	Relative dry weight	Dry weight	Relative dry weight
	Number	*Grams*	*Percent*	*Grams*	*Percent*
Control	4	1,134	100	2,717	100
50-chloride	4	613	54	1,720	63
150-chloride	4	80	7	421	15
50-sulfate	4	961	85	2,213	81
150-sulfate	4	399	35	1,165	43
250-sulfate	4	150	13	586	22

ALFALFA, CHILEAN (APR. 17-OCT. 10)

Nutrient solution	Plants surviving	Hay cuttings								Roots	
		Dry weight				Relative dry weight				Dry weight	Relative dry weight
		July 10	Aug 21	Oct 30	Total	July 10	Aug 21	Oct 30	Total		
	Number	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	*Percent*
Control	15	385	201	235	821	100	100	100	100	87	100
50-chloride	13	235	158	210	603	61	79	89	73	81	93
150-chloride	11	137	107	114	358	36	53	49	44	63	72
50-sulfate	15	276	196	189	661	72	98	80	81	67	77
150-sulfate	15	232	162	176	569	60	81	75	69	62	71
250-sulfate	15	178	153	135	466	46	77	57	57	54	62

COTTON, ACALA (APR. 17-NOV. 9)

Nutrient solution	Plants tested	Seed cotton		Entire plants	
		Dry weight	Relative dry weight	Dry weight	Relative dry weight
	Number	*Grams*	*Percent*	*Grams*	*Percent*
Control	4	622	100	2,116	100
50-chloride	4	469	75	1,560	74
150-chloride	4	287	46	870	41
50-sulfate	4	492	79	1,321	62
150-sulfate	4	460	74	1,274	60
250-sulfate	4	251	40	657	31

TOMATO, STONE (APR. 17-SEPT. 20)

Nutrient solution	Plants tested	Vines (excluding fruit)		Total fruits					Entire plants	
		Dry weight	Relative dry weight	Fresh weight	Dry weight [1]	Relative dry weight	Average fresh weight (per fruit)	Number with blossom-end rot	Dry weight	Relative dry weight
	Number	*Grams*	*Percent*	*Grams*	*Grams*	*Percent*	*Grams*	*Percent*	*Grams*	*Percent*
Control	2	2,232	100	16,741	921	100	130	0	3,153	100
50-chloride	2	1,719	77	13,619	749	81	90	7	2,468	78
150-chloride	2	594	27	673	37	4	22	34	631	20
50-sulfate	2	1,764	79	12,115	666	72	148	2	2,430	77
150-sulfate	2	1,363	61	4,480	246	27	43	78	1,609	51
250-sulfate	2	771	35	1,774	98	11	25	84	869	28

[1] Assumed moisture content of fresh fruit, 94.5 percent.

TABLE 3.—*Growth and yield of plants in experiment 1*—Continued

BARLEY, CLUB MARIOT (APR 17–JULY 31)

Nutrient solution	Plants		Grain		Entire plants	
	Tested	Average height	Dry weight	Relative dry weight	Dry weight	Relative dry weight
	Number	*Centimeters*	*Grams*	*Percent*	*Grams*	*Percent*
Control	12	100	[3] 214	100	564	100
50-chloride	12	88	295	138	685	121
150-chloride	12	67	104	49	219	39
50-sulfate	12	90	247	115	495	88
150-sulfate	12	75	144	67	322	57
250-sulfate	12	70	67	31	195	35

SUGAR BEETS, U S NO. 1 (APR. 17–OCT. 29)

Nutrient solution	Plants tested	Beets	
		Average fresh weight	Average fresh weight (relative)
	Number	*Grams*	*Percent*
Control	4	1,475	100
50-chloride	4	1,452	98
150-chloride	4	1,281	87
50-sulfate	4	1,148	78
150-sulfate	4	1,209	82
250-sulfate	4	837	59

[3] The control barley, though systematically dusted, was heavily infested with mildew There was little or no mildew on the other barley plants. The early growth of the control barley was more rapid than that of the other barley plants.

The lemon plants, which were from 4-inch January cuttings with two leaves, were rooted in the greenhouse in coarse sand and transplanted when the first growth cycles were making their appearance. Of the eight crops, lemon was the most sensitive to the chloride salts. The combined roots, stems, and retained leaves of the three plants supplied with solution containing 50 milliequivalents of chloride per liter weighed 28 percent as much as those of the plants in the control bed, and all of the plants in the 150-chloride bed died. The sulfate tolerance of the lemon plant was much higher than its chloride tolerance; two of the three rooted cuttings set out in the 250-sulfate bed lived until the end of the season, all of them making some growth.

Chapman and Liebig (5), in greenhouse experiments, found no depression in the growth of sweet orange seedlings from either 20 milliequivalents of chloride or 20 milliequivalents of sulfate when these ions were added as mixed salts of potassium, calcium, and magnesium in a series of solutions of different nitrate concentration.

Milo was substantially more tolerant to chloride during its early growth stages than after the approach of flowering. With the appearance of the flower stalks the leaves of plants in the 150-chloride bed began to burn, and they made poor heads, whereas the burning of milo leaves in the sulfate beds came on gradually. This observation, which was again made in a later experiment, points to a chloride-induced physiological upset associated with the time of rapid translocation of materials to the flower stalks in this variety of milo. It will later be shown (tables 5 and 6) that milo in the 150-chloride bed accumulated a disproportionately high concentration of chloride ion in its sap.

Three cuttings were made from the alfalfa, and there was considerable variation in the growth depression of these successive crops in

both the chloride and the sulfate series. The growth of the midsummer crop was reduced relatively less by salt than either the first or final crop.

The vegetative growth of cotton plants was reduced relatively more by chloride and sulfate salts than was the yield of seed cotton. The average weight of individual bolls was not affected by the treatments. The plants from this experiment are shown as figure 1.

Fruit production by tomatoes was depressed by salt roughly in proportion to the respective reductions in vine growth in the 50-chloride and 50-sulfate beds, but in the beds maintained with higher salt concentrations blossom-end rot became the dominant factor in yield. Blossom-end rot and its greater severity in the sulfate beds will receive consideration after the discussion of the analysis of the expressed sap.

The barley in the control bed was severely affected with mildew, but there was little or none on the plants in the salt beds. Because of the poor condition of the control barley, satisfactory conclusions cannot be drawn with respect to this plant's salt tolerance. It may be noted, however, that the reductions in yield between the 50- and 150-chloride and between the 50-, 150-, and 250-sulfate beds are proportionately much sharper than was the case with some other crops, indicating that mildew had substantially reduced the growth of the control plants. It has been observed, both in .this experiment and in other experiments at this laboratory, that either chloride or sulfate salt, as well as boron (8), serves to repress mildew infestations of barley.

The sugar beets showed a high degree of tolerance to chloride but not to sulfate salts; a greater reduction in yield was found to result from 50 milliequivalents of sulfate than from 150 milliequivalents of chloride.

Lill, Byall, and Hurst (16) have recently reported an increased growth of sugar beets in Michigan following applications of upward to 1,000 pounds of sodium chloride per acre. These increases were not as great as those resulting from complete fertilizers, and they were attributed to the indirect effects of the salt in the liberation of nutritional elements from the soil.

To facilitate a comparison of the toxicity of chloride and sulfate salts to this series of plants, the means of the relative production in the 50- and 150-sulfate beds and in the 150- and 250-sulfate beds have been set opposite the relative yields in the 50-chloride bed (table 4). Under the conditions of this experiment, 50 milliequivalents of chloride brought about growth depressions of dwarf milo, alfalfa, and cotton that were roughly equal to those indicated for 100 milliequivalents of sulfate. The toxicity of chloride to these particular plants, as measured in milliequivalents, was thus about twice as great as the toxicity of sulfate. Navy beans withstood 100 milliequivalents of sulfate substantially better than 50 milliequivalents of chloride, and the lemon plants withstood three or four times as much sulfate ion as chloride ion. Beets, on the other hand, were nearly as sensitive as alfalfa to sulfate, but they withstood high concentrations of chloride with little injury. The tomato plants withstood 50 milliequivalents of chloride better than 100 milliequivalents of sulfate.

On the basis of the similarity in the toxicity of 50 milliequivalents of chloride and 100 milliequivalents of sulfate to milo, alfalfa, and

TABLE 4.—*Growth of plants in 50 milliequivalents of chloride and in 100 and 200 milliequivalents of sulfate (average of 50 and 150 milliequivalents and 150 and 250 milliequivalents of sulfate) relative to the control plants*

Crop	Yield [1] with indicated concentration of—		
	Chloride	Sulfate	
	50 milliequiv- alents	100 milliequiv- alents	200 milliequiv- alents
	Percent	*Percent*	*Percent*
Lemon cuttings	28	59	24
Navy beans (seed)	39	45	
Dwarf milo (grain)	54	60	24
Chilean alfalfa (3 cuttings)	73	75	63
Acala cotton (seed cotton)	75	77	57
Stone tomatoes (entire)	78	54	40
Sugar beets (fresh roots)	98	80	71

[1] Percentage of yield with control nutrient solution.

cotton, it might be urged that if the concentrations of the chloride and sulfate salts had been measured in terms of electrical conductivity or freezing-point depression (see table 5) or in terms of moles of salt or of total solids, an equal toxicity of the two ions would have been indicated. The possibility of such simple relationships in salt toxicity is largely eliminated, however, when account is taken of the fact that two of these seven crops (lemon and navy bean) were far more tolerant of 100 milliequivalents of sulfate than of 50 milliequivalents of chloride and that two others (tomato and sugar beet) were substantially more tolerant of 50 milliequivalents of chloride than of 100 milliequivalents of sulfate. The evidence of specific ion effects is such as to indicate that it will not be possible to satisfactorily evaluate chloride and sulfate toxicity on the basis of any of these summation indices. Furthermore, the toxicity of chloride and sulfate cannot be expected to be independent of the kind and proportions of the bases with which they are associated in culture or soil solutions.

SYMPTOMS

The leaves of lemon plants in the control bed were larger than those in the salt beds, and the control plants were more vigorous and healthy in appearance. Nevertheless, symptoms of diagnostic value were lacking except for some yellowing, which preceded leaf abscission in both the chloride and sulfate beds and an occasional burned tip in the chloride bed. Badly injured lemon trees in San Diego County, Calif., irrigated with a water high in chloride, have been observed to show marked bronzing of the leaves, and the fruit from the grove softened badly while in the packing house. Chapman and Liebig (5) record some burning of the leaves of their orange seedlings in 20 milliequivalents of chloride, but this was not accompanied by reduced growth.

The size of the leaves of the beans in the salt beds was reduced roughly in proportion to the reduction in plant size, but the size of the mature seeds was not affected. When harvested on July 13, the control plants and the plants in the 50-sulfate bed had a few dead leaves, but nearly all were green. Most of the leaves had burned and if still retained were nearly ready to drop from the plants in the 50-chloride and 150-sulfate beds.

There were outstanding differences at time of harvest in the amount

of burning of the milo leaves under the different treatments.   At this stage the oldest leaves of the control plants had already died, and the margins of the leaves successively higher up the stalk showed drying to a decreasing degree; in the 50-chloride bed, from 25 to 50 percent of the leaf tissue was dead; the drying started at the margin, but it was accompanied by some yellow striping between the veins.   The milo in the 150-chloride bed was practically dead when harvested a month in advance of the five other cultures.   Less than 25 percent of the leaf tissue was dead in the 50-sulfate milo bed, and about 50 percent in the 250-sulfate; the plants in the 150-sulfate bed were intermediate.   The stalks of the plants in all of the beds except the 150-chloride were still green when harvested, but they had started to dry near the heads.

Except for a reduction in plant size and a tendency toward slightly smaller leaves, the alfalfa in the high-chloride and high-sulfate beds lacked outward leaf symptoms indicative of salt injury.   The margins of some leaves in the salt beds turned white in a narrow band, but this symptom cannot be regarded as specific, since it has been observed in fields and in other experiments where high salt conditions did not exist.   The last two crops of alfalfa began to flower irregularly earlier in the chloride- and sulfate-treated beds than in the control bed.

The cotton plants growing in the high-chloride and high-sulfate beds were reduced in size, and the leaves were somewhat smaller than the controls, but otherwise all were normal in appearance (fig. 1).   Symptoms of injury or other abnormalities were lacking.

FIGURE 1.—Cotton grown in the 1934 sand-culture experiments, with various concentrations of salts added to the nutrient solution: *A*, Control; *B*, 50 milliequivalents of chloride per liter; *C*, 150 milliequivalents of chloride; *D*, 50 milliequivalents of sulfate; *E*, 150 milliequivalents of sulfate; and *F*, 250 milliequivalents of sulfate.

Aside from the prevalence of blossom-end rot on the tomatoes, neither chloride nor sulfate salts produced symptoms of injury.

The tips of older leaves of barley plants under both the chloride and sulfate treatments were burned considerably, and this burning was more pronounced with the higher concentrations.

The leaves of the beets in all beds were normal in appearance.

GROWTH-DEPRESSION GRAPHS

The character of the growth-depression graphs (fig. 2) is regarded as significant. No evidence is afforded by these graphs of a particular range of chloride or sulfate concentrations that can be regarded as critical in the sense that the term is sometimes applied in discussions of salt toxicity. Starting at some most favorable concentration, undetermined in this experiment, each additional unit of chloride and sulfate brought about further depression in growth. The graphs tend to flatten out as the concentrations become higher, indicating that the toxicity per unit of salt decreased as units were added. This latter
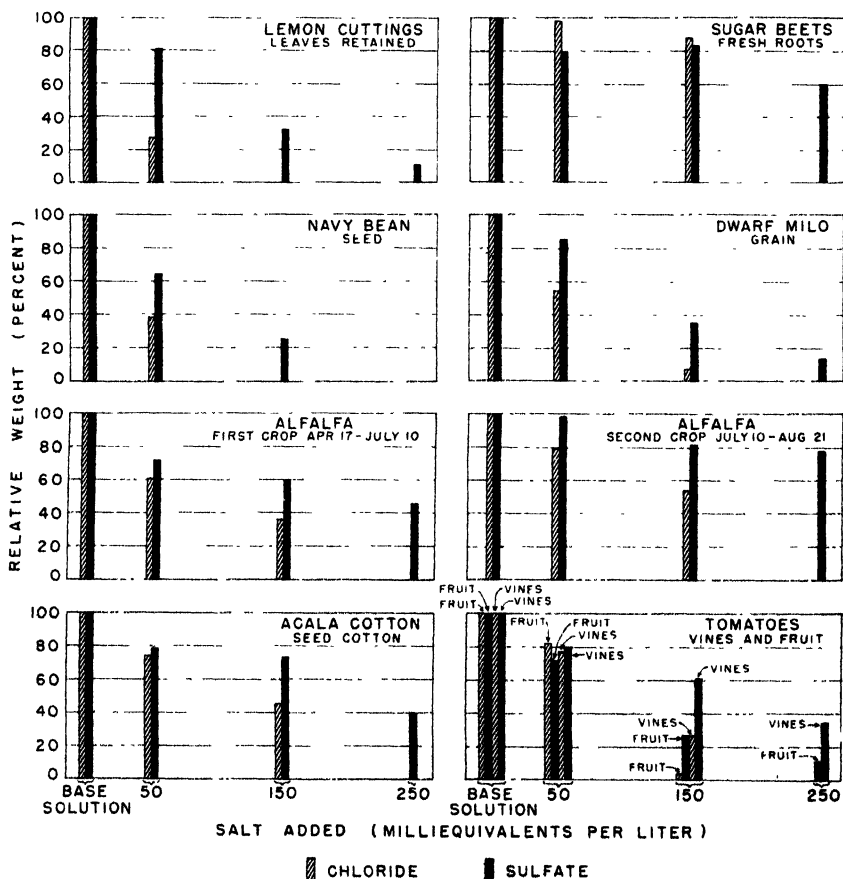
FIGURE 2.— Growth depression produced by chloride and sulfate salts. The parts of plants taken are as indicated under the names of the crops. Growth is represented relative to that of plants in the control bed.

point will be taken up again in the discussion of the analyses of plant saps and in connection with experiments conducted in water cultures in the greenhouse.

PHYSICAL AND CHEMICAL PROPERTIES OF PLANT SAP

EXTRACTION OF PLANT SAP

The samples of freshly picked leaves, or entire tops in the case of alfalfa and barley, were compacted in glass tubes, which were then stoppered and sealed with paraffin paper secured in place with rubber bands. These tubes were packed in solid carbon dioxide and allowed to stand overnight, after which the samples were thawed out one at a time, and the sap was expressed while still cold by the gradual application of pressure of 2,400 pounds per square inch in a Carver press having monel-metal parts. The samples were left at this pressure for several minutes or until the rate of sap release became very slow.

The evidence of Phillis and Mason (*26*) on the marked differences in the composition of the fluids expressed from flat piles of unfrozen cotton leaves (regarded as vacuolar sap) and that obtained after the residue was frozen (regarded as principally cytoplasmic sap) raises important questions as to the equilibriums existing between these leaf-tissue fluids and the extent of dissociation of the organic and inorganic materials in living cytoplasm. This question of interpretation and expressed-sap data extends further to possible differences in composition and physical characteristics between the fluids obtained from palisade, spongy parenchyma, and epidermal tissues, all of which, as shown by Turrell (*33*), are extensively exposed to the intercellular air spaces of the leaf. Leaving these problems and the indicated complex relations as they now stand, there still remains much justification for employing the customary methods in studies of the reaction of plants to their environment in terms of the characteristics of the composite sap obtained from frozen tissue.

EFFECT OF SALT ON SUCCULENCE

Barley was the only crop that showed a change in quantity of expressed sap with changes in the composition of the nutrient solution; both chloride and sulfate salts decreased the succulence of barley. The control and the barley plants in the 50- and 150-chloride beds yielded respectively 72, 48, and 39 ml. of sap per 100 gm. of fresh tissue, and the control and the plants in the 50-, 150-, and 250-sulfate beds yielded respectively 72, 51, 45, and 32 ml. The quantities of expressed sap from the other plants were notably uniform throughout the treatments. The average yield of sap from milo leaves was 52 ml. per 100 gm. of tissue; from alfalfa plants, 47 ml.; from cotton leaves, 67 ml.; from tomato leaflets, 71 ml.; and from sugar-beet leaf blades, 47 ml.

ACCUMULATION OF SALT CONSTITUENTS IN PLANT SAP

Determinations were made of the concentration of some or all of the following constituents in each of the sap samples: Calcium, magnesium, sodium, potassium, total sulfur, chloride, and total phosphorus. The official methods of the Association of Official Agricultural Chemists for plant materials were followed, with the exception that sodium was determined by the uranyl-zinc-acetate method and potassium by a cobaltinitrite procedure. The data on

date of collection of the samples and the parts of the plants represented are given in table 8. For purposes of discussion total sulfur and total phosphorus are treated as SO$_4$ and PO$_4$.

The results of the sap analyses and the average composition of the nutrient solutions are presented in tables 5 and 6. In table 5 each crop is considered in turn and the effect of the added salts is shown on the pH value, electrical conductance, and ionic concentration. In table 6 the data are so grouped as to bring out differences among the crops in the accumulation of each of the electrolytes. The first method is the more logical, but the second furnishes a better basis for discussion of the comparative reactions of the different crops. In table 6 the accumulation ratios also are shown. The term "accumulation ratio," as here used, signifies the concentration of a constituent in expressed plant sap divided by the concentration in the supporting nutrient solution, i. e., the concentration gradient against which additional small quantities of the ion would be accumulated. Eight different crops were grown in the beds, but there was insufficient material for sap analyses of the lemons and navy beans, and only partial analyses were made of the barley. The agricultural plants used for the salt-uptake comparisons (table 5) represent five well-differentiated botanical families, namely, the Gramineae (two representatives), the Leguminosae, the Malvaceae, the Solanaceae, and the Chenopodiaceae.

Certain important similarities of reaction to the added salts have been found among these six crop plants, but on first appraisal diversity of behavior and species specificity are the prominent features. The data well illustrate the hazards associated with the formulation of generalizations on ionic uptake and interionic effects on the basis of the reactions of a single plant species.

All crops accumulated relatively high concentrations of cations from the base nutrient solution (table 6), but the concentrations in milo, for example, were only approximately one-half as great on all solutions as those in cotton. The concentration of total bases in the sap of milo and cotton in the control bed was, respectively, 18.2 and 34.8 times as great as in the nutrient solution. Alfalfa, tomatoes, and sugar beets occupied intermediate positions. The total concentration and, in most instances, the concentration of individual ions in the sap were greater in plants grown in the salt beds than in the control bed, but it is to be noted that the accumulation ratios decreased with the addition of salt. The milo and cotton, with accumulation ratios of 18.2 and 34.8, respectively, for total bases in the control bed, had ratios of 1.8 and 3.5, respectively, in the 250-sulfate bed.

The sharp decreases in accumulation ratios with the addition of ions to the nutrient have shown the need of an additional measure of the effects of substrate concentrations upon the accumulation of salt in plants. To this end there are set forth in table 7 what are termed "increment ratios." These values represent the ratios of the differences in the concentration of the ions in the sap of the salt-treated and the control plants divided by the differences between the saline and control solutions:

$$\text{Increment ratio} = \frac{\text{sap concentration of salt-treated plant} - \text{sap concentration of control plant}}{\text{solution concentration in salt bed} - \text{solution concentration in control bed}}$$

TABLE 5.—*Concentration of inorganic constituents in expressed plant sap and in supporting nutrient solutions of various crops (experiment 1, 1934)*

Nutrient solution and source of sap	pH	Freez-ing-point depres-sion (°C.)	Con-duct-ance (K×10⁵ at 25° C.)	Ca	Mg	Na	K	Sum	Cl	Total S as SO₄	Total P as PO₄	NO₃
Culture solutions (average new and discarded)												
Control	7.2	0 06	172	5 49	2 26	3 78	5.84	17 4	0.58	2.68	¹ 0.15	7.79
50-chloride	7 2	.21	665	17 72	14.73	27 40	6 81	66.7	51.21	2 93	.13	8.58
150-chloride	7 1	.50	1,620	42 44	41.56	77 70	8 06	169.8	149.33	3 26	15	11 03
50-sulfate	7 1	.15	531	16 41	15 29	25.55	7 01	64.3	90	50 02	.14	8.34
150-sulfate	7 2	.29	1,006	23 58	58 50	73 55	7. 56	163 3	.74	147 47	.18	9 43
250-sulfate	7.2	42	1,603	25 14	105 40	120 43	8 02	259	.82	241 29	.29	11 08
Milo, dwarf (leaves)												
Control	4 72	.92	1,690	63	74	1	179	316	26	21		
50-chloride	4 63	1 11	1,868	96	144	4	155	399	81			
150-chloride	4 38	1 49	2,731	151	210	14	180	555	267			
50-sulfate	4 68	1 04	1,732	57	100	5	180	342	35			
150-sulfate	4 57	1 10	1,821	44	179	9	194	426	27	45		
250-sulfate	4 56	1 15	1,821	42	216	13	203	474		110		
Alfalfa (first cutting)												
Control	5 31	.95	1,628	125	16	8	167	316		47		
50-chloride	5 25	1 06	1,776	158	47	14	112	331		27		
150-chloride	5 13	1 28	2,093	151	69	31	109	360		43		
50-sulfate	5 28	1.03	1,724	109	55	15	149	328		49		
150-sulfate	5 25	1 20	1,953	88	96	18	164	366		82		
250-sulfate	5 32	1 41	1,831	71	142	35	163	411	15	142		
Alfalfa (second cutting)												
Control		1 26	2,284	128	68	15	342	551				
50-chloride		1 26	2,372	121	76	32	306	535	84			
150-chloride		1.41	2,128	118	94	32	297	541	130			
50-sulfate		1.15	2,097	80	64	32	286	462	21			
150-sulfate		1 14	1,891	56	89	58	250	453	19			
250-sulfate		1 37	2,156	55	159	80	240	534	17			
Alfalfa, third cutting (stems and leaves)												
Control		1 23	2,149	107	62	25	265	459	25	38	33	
50-chloride		1.28	2,324	109	74	39	250	472	79	31	30	
150-chloride		1 47	2,753	133	98	65	230	526	171	33	33	
50-sulfate		1 27	2,135	90	70	53	255	468	27	45	30	
150-sulfate		1 31	2,179	58	100	81	243	482	20	67	42	
250-sulfate		1 43	2,359	49	156	121	224	550	17	118	46	
Cotton, Acala (leaves)												
Control		1 15	2,024	251	108	18	228	605	18	183	7	
50-chloride		1 19	2,211	335	134	26	185	680	76	230	7	
150-chloride		1 31	2,716	427	165	27	132	751	178	232	5	
50-sulfate		1 15	2,165	358	141	25	255	779	9	328	7	
150-sulfate		1. 16	2,382	352	223	38	208	821	4	392	9	
250-sulfate		1 23	2,692	334	283	51	240	908	2	439	9	
Tomato (leaves)												
Control		.79	1,886	115	83	6	161	365	25	150	26	
50-chloride		.90	2,179	164	130	11	129	434	71	152	20	
150-chloride		1 18	2,724	205	130	14	154	503	155	128	24	
50-sulfate		89	2,101	89	111	15	177	392	23	177	24	
150-sulfate		1.01	2,147	315	68	17	165	565	17	222	27	
250-sulfate		1 14	2,354	478	63	28	153	722	13	209		
Barley (stems, leaves, and flowering heads)												
Control	6 01	.83	2,438						63	16		
50-chloride	5 99	1.24	2,882						168	21		
150-chloride	5 66	1.72	3,963						347	22		
50-sulfate	6 00	1.50	2,642						89	26		
150-sulfate	6 02	1 39	3,019						96	73		
250-sulfate	6 02	1.57	3,170						91	116		
Sugar beets (leaves):												
Control	5 97	1 13	2,964	(²)	101	229	167	497	44	40	23	
50-chloride	5.95	1 36	3,392	(²)	145	297	192	634	91	29	17	
150-chloride	5.96	1.75	4,126	(²)	167	392	187	746	175	41	17	
50-sulfate	5.93	1.31	3,180	(²)	103	258	190	551	31	55	17	
150-sulfate	5.95	1.41	3,318	(²)	130	370	120	620	43	82	15	
250-sulfate	5. 99	1. 40	3,550	(²)	104	347	158	609	44	110	13	

¹ Average of used solutions only. There was a heavy precipitation of phosphate when new solutions were first applied.
² Trace.

The concentrations of constituents in the sap of plants growing in the salt beds were in some instances found to be lower than those in the sap of control plants; these reductions in concentration are indicated by —c. Ratios below unity show that the sap concentration of the salt plant was greater than that of the control plant but that the

TABLE 6.— *Concentrations and accumulation ratios of inorganic constituents in nutrient solutions and plant saps*

Treatment	Control bed	(A) Concentration (milliequivalents per liter) Chloride bed		Sulfate bed			Control bed	(B) Accumulation ratio (sap concentration/solution concentration) Chloride bed		Sulfate bed		
		50 milli-equivalents	150 milli-equivalents	50 milli-equivalents	150 milli-equivalents	250 milli-equivalents		50 milli-equivalents	150 milli-equivalents	50 milli-equivalents	150 milli-equivalents	250 milli-equivalents
Total cations:												
Culture solution	17 4	66 7	169.8	64 3	163 3	259						
Expressed sap:												
Milo	316	399	555	342	426	474	18 2	6 0	3 3	5 3	2.6	1.8
Alfalfa (third cutting)	459	472	526	468	482	550	26 4	7 1	3 1	7 3	3 0	2.1
Cotton	605	680	751	779	821	908	34 8	10 2	4 4	12 1	5.3	3 5
Tomato	365	434	503	392	565	722	21.0	6 5	3.0	6 1	3 5	2 8
Sugar beet	497	634	746	551	620	609	28 6	9.5	4 4	8 6	3 8	2 4
Calcium:												
Nutrient solution	5 5	17 7	42 4	16 4	23.6	25 1						
Expressed sap:												
Milo	63	96	151	57	44	42	11 5	5 4	3 6	3 5	1 9	1 7
Alfalfa (third cutting)	107	109	133	90	58	49	19 5	6 2	3 1	5 5	2.5	2.0
Cotton	251	335	427	358	352	334	45 6	18 9	10 1	21 8	14.9	13 3
Tomato	115	164	205	89	315	478	20 9	9 3	4.8	5.4	13 3	19.0
Sugar beet	(¹)	(¹)	(¹)	(¹)	(¹)	(¹)						
Magnesium:												
Nutrient solution	2 3	14 7	41 6	15.3	58 6	105.4						
Expressed sap:												
Milo	74	144	210	100	179	216	32 2	9 8	5 0	6 5	3 1	2 0
Alfalfa (third cutting)	62	74	98	70	100	156	27.0	5 0	2 4	4 6	1 7	1 5
Cotton	108	134	165	141	223	283	47 0	9 1	4 0	9.2	3 8	2 7
Tomato	83	130	130	111	68	63	36 1	8 8	3 1	7.3	1 2	6
Sugar beet	101	145	167	103	130	104	43.9	9.9	4 0	6 7	2 2	1 0
Sodium:												
Nutrient solution	3 8	27 4	77 7	25 6	73.6	120 4						
Expressed sap:												
Milo	1	4	14	5	9	13	.3	.2	2	2	1	.1
Alfalfa (third cutting)	25	39	65	53	81	121	6 6	1 4	8	2 1	1.1	1 0
Cotton	18	26	27	25	38	51	4.7	1 0	4	1 0	.5	.4
Tomato	6	11	14	15	17	28	1 6	4	2	6	2	2
Sugar beet	229	297	392	258	370	347	60 3	10.8	5 0	10 1	5 0	2 9
Potassium:												
Nutrient solution	5 8	6 8	8 1	7.0	7 6	8.0						
Expressed sap:												
Milo	179	155	180	180	194	203	30 9	22 8	22 2	25 7	25.7	25.4
Alfalfa (third cutting)	265	250	230	255	243	224	45 7	36.8	28 4	36 4	32 0	28 0
Cotton	228	185	132	255	208	240	39 3	27.2	16 3	36 4	27 4	30 0
Tomato	161	129	154	177	165	153	27 8	19.0	19 0	25 3	21 7	19 1
Sugar beet	167	192	187	190	120	158	28 8	28 2	23 1	27.1	15.8	19.8
Chloride:												
Nutrient solution	6	51 2	149 3	9	.7	.8						
Expressed sap:												
Milo	26	81	267	35	27		43 3	1.6	1.8	38 9	38.6	
Alfalfa (third cutting)	25	79	171	27	20	17	41.7	1.5	1 1	30.0	28.6	21.3
Cotton	18	76	178	9	4	2	30.0	1.5	1 2	10 0	5 7	2.5
Tomato	25	71	155	23	17	13	41.7	1 4	1.0	25 6	24.3	16.3
Sugar beet	44	91	175	31	43	44	73.3	1.8	1.2	34.4	61.4	55.0
Sulfate:												
Nutrient solution	2.7	2 9	3.3	50	147 5	241.3						
Expressed sap:												
Milo	21				45	110	7.8				.3	.5
Alfalfa (third cutting)	38	31	33	45	67	118	14.1	10.7	10 0	.9	.5	.5
Cotton	183	230	232	328	392	439	67.8	79 3	70.3	6.6	2.7	1.8
Tomato	150	152	128	177	222	299	55.6	52.4	38.8	3 5	1.5	1.2
Sugar beet	40	29	41	55	82	110	14.8	10.0	12.4	1.1	.6	.5

¹Trace.

increment was less than the quantity added to the base nutrient. Values above unity show the extent to which sap accumulations exceeded the additions to the solutions.

In contrast to the high accumulation ratios found in the control bed, sodium excepted, the increment ratios are usually low; the

TABLE 7.—*Concentrations and increments of ions in culture solutions, and corresponding accumulation ratios and increment ratios* [12] *in sap of crop plants (experiment 2)*

Ion and nutrient solution	Culture solution		Milo		Alfalfa		Cotton		Tomato		Sugar beet	
	Concentration	Increment	Accumulation ratio	Increment ratio	Accumulation ratio	Increment ratio	Accumulation ratio	Increment ratio	Accumulation ratio	Increment ratio	Accumulation ratio	Increment ratio
	Milli-equivalents	*Milli-equivalents*										
Total cations.												
Control	17.4		18.2		26 4		34 8		21 0		28.6	
50-chloride		49.3		1 7		0.3		1.5		1.4		2.8
150-chloride		152 4		1 6		.4		1.0		.9		1.6
50-sulfate		46 9		6		.2		.4		6		1 2
150-sulfate		145 9		.8		.2		1 5		1 4		.8
250-sulfate		241.6		.7		.4		1 3		1.5		.5
Calcium												
Control	5 5		11.5		19 5		45 6		20 9			
50-chloride		12 2		2 7		.2		6.9		4 0		
150-chloride		36 9		2 4		.7		2 5		2 4		
50-sulfate		10 9		-c		-c		9 8		-c		
150-sulfate		18 1		-c		-c		5 6		11 1		
250-sulfate		19 6		-c		-c		4 2		18.5		
Magnesium.												
Control	2 3		32.2		27 0		47 0		36 1		43 9	
50-chloride		12 4		5 7		1 0		2 1		3 8		3 6
150-chloride		39 3		3.5		.9		1 5		1 2		1 7
50-sulfate		13 0		2 0		6		2.5		2 2		.2
150-sulfate		56.3		1 9		.7		2.0		-c		.5
250-sulfate		103.1		1 4		.9		1 7		-c		0
Sodium·												
Control	3 8		.3		6 6		4 7		1 6		60 3	
50-chloride		23 6		.1		6		.3		.2		2.9
150-chloride		73 9		2		5		.1		1		2 2
50-sulfate		21.8		2		1.3		.3		.4		1 3
150-sulfate		69.8		1		8		.3		2		2 0
250-sulfate		116.6		.1		.8				2		1.0
Chloride												
Control	.6		43 3		41 7		30 0		41 7		73 3	
50-chloride		50 6		1.1		1 1		1 2		.9		.9
150-chloride		148.7		1 6		1.0		1 1		9		.9
50-sulfate		.3									
150-sulfate		.1									
250-sulfate		2									
Sulfate												
Control	2.7		7.8		14.1		67 8		55.6		14.8	
50-chloride		.2									
150-chloride		6									
50-sulfate		47 3				2		3 1		.6		.3
150-sulfate		144.8		.2		2		1 4		.5		.3
250 sulfate		238.6		.4		3		1 1		.6		.3

[1] Increment ratio = $\dfrac{\text{sap concentration of salt-treated plant} - \text{sap concentration of control plant}}{\text{solution concentration in salt bed} - \text{solution concentration in control bed}}$

[2] —c indicates a concentration in sap of salt plant less than that in sap of control plant.

values are more often below unity than above. Except for the sugar beets in all salt beds and alfalfa in the 50-sulfate bed, no plant increased the sodium concentration in its sap by as much as the solution was increased. Noteworthy is the fact that the chloride increments in the sap of all plants in the two chloride beds were approximately equal to the increments added to the solutions; except for the milo in the 150-chloride bed, no chloride-increment ratio exceeded 1.2

and none fell below 0.9. In other words, with the addition of either 50 or 150 milliequivalents of chloride to the base solution, which contained 0.6 milliequivalent, each of these five botanically distinct plants increased the concentration of chloride in its sap by quantities nearly equal to the additions to the culture solutions. In other experiments, not included in this report, chloride-increment ratios that departed further from unity have been observed, and climatic variables may have had an important influence. The growth of most of these plants was markedly reduced with these higher chloride levels in the sap, but the fact that the growth of beets was affected but little is to be especially noted as an indication of the great diversity in the responses of different plants to similar accumulations. The relatively low increment ratios for nearly all ions and plants as here found probably constitute one of the most important points to be considered in any study of the reactions of plants on saline substrates.
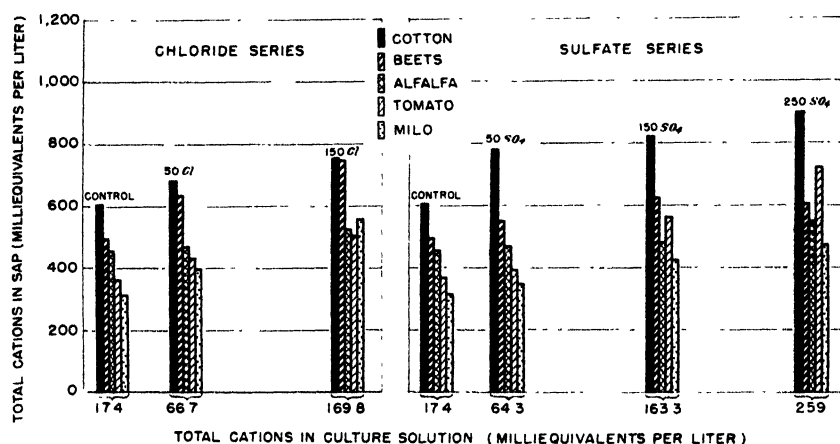
FIGURE 3.— Accumulation of total bases in the sap of plants grown in the control solution and in the chloride and sulfate solutions.

In figure 3, total bases in the plant saps are plotted against the total bases in the corresponding culture solutions. The concentration of total bases in the nutrient solution of the control bed was 17.4 milliequivalents per liter. The tenfold increase in the concentration of bases in the solution serving the high-chloride bed or the fourteen-fold increase in the high-sulfate bed in no instance served to double the concentrations of total bases in the expressed sap of any of these plants.

When the accumulations of the individual bases in table 6 are examined, much specificity is found to exist. The sap of the sugar beet, for example, in harmony with the observation of McCool and Weldon (*20*), contained little or no calcium, although this plant has not been found abnormal when calcium determinations were made on dry leaf tissue. Cotton and tomatoes, on the other hand, tend to maintain a high calcium concentration. The tomato plants accumulated far more calcium from solutions high in chloride than from those high in sulfate, whereas with cotton the opposite relationship was found. Calcium accumulation is thus not independent of the kind of anion with which it is supplied.

The evidence of a correlation between accumulation of calcium and magnesium, which Collander (*6*) mentions, is least apparent in the sap of this series of plants; calcium and magnesium accumulations do tend to parallel one another in the chloride series, though in different amounts, but in the sulfate series an accumulation of one is most often associated with a depression of the other. Comparisons of the sap of the five species of plants grown in the control bed show little correlation between their calcium and magnesium content.

A notably wide variation is shown by different plant species in the extent to which they accumulate sodium. The sodium accumulation ratios (table 6, *B*) of milo, alfalfa, cotton, and tomatoes were very low. The beet, on the other hand, accumulated sodium in high concentrations under all the treatments. Sodium uptake was influenced by the concentration of sodium in the culture solutions, and it made little difference whether it was added as the chloride or sulfate salt. There is little if any evidence of interrelations between sodium and potassium accumulation, but in more recent work with barley sodium accumulation has been found to depress potassium accumulation. Van Itallie (*34*) found little sodium in the sap of wheat and potatoes and much in the sap of mustard and mangels. Collander (*6*) also found a great diversity in sodium uptake among plants.

It is to be observed that only a few major changes in the accumulation of potassium in the sap of these plants resulted from upward to an eightfold increase in the concentration of calcium in the nutrient solution, a forty-fivefold increase in magnesium, a thirtyfold increase in sodium, a 250-fold increase in chloride, or a ninetyfold increase in sulfate. The most important exception was the depression in potassium uptake by the cotton grown in the 50-chloride and 150-chloride beds, but no such depression occurred from the sulfate additions. The uniformity of potassium uptake as between crops is likewise noteworthy when account is taken of the highly preferential absorption shown by these crops for different ones of the other ions present in solution. Van Itallie (*34*) has recently pointed to a similar uniformity in potash accumulation by a series of eight crops grown on a soil with and without sodium and sodium plus potassium additions. Van Itallie's results, which were all referred to the nitrogen found in the tissues, showed a marked variability between plants in the accumulation of cations other than potassium. Collander (*6*) likewise found among plants greater uniformity in potassium than in sodium or calcium accumulations.

With an average concentration of 0.6 milliequivalent of chloride in the base nutrient solution of the control bed (table 6), from 18 to 44 milliequivalents was found in the sap of the five crops. With the addition of 50 and 150 milliequivalents of chloride to the solution there tended to be equivalent increases in the sap concentration. In other words, the increments in the sap tended to equal the increments in the solution. Milo in the 150-chloride bed was an exception.

Cotton and tomatoes throughout all treatments carried in their sap several times as much sulfur as milo, alfalfa, or sugar beets. The concentration of sulfur found in the sap of cotton in the high-sulfate bed corresponds to that of a 0.44-normal sulfate solution.

Phosphate determinations were made on only four sets of sap samples. These data show that between plants such as alfalfa and cotton (table 5) there are substantial differences in phosphate con-

centrations, but these concentrations were not influenced to any major extent by the uptake of other ions. The accumulation ratios were in all cases relatively high.

The pH values of the sap of some of these crops were lower for plants grown on solutions high in chloride than for those grown in the control bed; the effect is shown by milo, alfalfa, and barley, but no change was shown by beets. Sulfate had little if any effect on the hydrogen-ion concentration in the sap. All of the pH measurements were made with a glass electrode.

In another experiment, dried samples of tomato leaves collected at the end of the growth period, when analyzed for chloride and sulfate and compared with the analyses of sap samples collected 11 days earlier, showed similar relative effects of the added salt on accumulation in the leaves. The concentrations of chloride found in the dried leaf blades were somewhat lower than in the petiole and stem tissues, but the concentrations of sulfur in the dried leaves were almost six times as great as in the combined stems and petioles.

ELECTRICAL CONDUCTIVITY OF PLANT SAP

The electrical conductivities of all of the sap samples are reported in table 5. It has long been known that the conductivity of a pure solution of an inorganic electrolyte is markedly influenced by the addition of an organic substance such as sugar, and that the interpretation of conductivities of plant sap in terms of concentrations of electrolytes is highly hazardous. Greathouse (*12*), in a recent paper on conductivity measurements of plant saps, has reviewed the conclusions of a number of investigators on this subject, adding additional material from his own investigations; he lists among factors affecting conductivity values of biological solutions such things as hydration of colloids and crystalloids (bound water), viscosity, ionic adsorption, and the surface conductance of colloids. The data here presented further illustrate the complex situation that is presented by conductivity measurements made on liquids expressed from frozen plant tissues.

As the concentration of nitrate in these expressed saps was not determined, the data of table 5 cannot be fully examined for correlations between total electrolytes and conductivity. Total cations, however, provide a basis for comparison since each cation must be balanced by an anion, either organic or inorganic. It is found that a fair degree of parallelism exists between conductivity and total cations through this series of sap samples, but an entirely different situation is presented when the conductivities and total cations of the saps are compared with those of the culture solutions. This effect of the organic constituents in warping conductivity values may be illustrated by comparing the electrical conductance ($1,620 \times 10^{-5}$) of the 150-chloride culture solution and that of the sap of the milo plants in the control bed, which had a conductance of $1,690 \times 10^{-5}$. The former had by analysis 169.8 milliequivalents of bases and the latter 316.

FREEZING-POINT DEPRESSION OF PLANT SAP AND DIFFERENTIALS BETWEEN SAP AND SUBSTRATE

Data on the freezing-point depressions of the culture solutions and of the expressed sap of this series of plants are included in table 5.

The diversity shown between species recalls to mind that found by Harris and his associates (*13*) among native species in each of a number of associations in the Tooele Valley, Utah.   In the control bed tomato was low, with a freezing-point depression of 0.79° C., and the second cutting of alfalfa was high, with a value of 1.26°.   The subject of primary interest in the present connection, however, has to do with the corresponding changes in the plant sap and the culture solutions.

With each addition of chloride or sulfate salt to the base nutrient solution there was in general an increase in the osmotic pressure of the expressed sap.   These increases in the freezing-point depression of the sap tended to parallel the corresponding increases in the freezing-point depression of the culture solutions (table 8).   In some instances the sap increases exceeded the increases in the solutions and in other instances they fell below.   If the six crops are viewed collectively, by means of averages, the gains in differentials are balanced by losses, which would indicate little basis for a generalization that plants on saline soils are at a disadvantage in their water relations.   The view has sometimes been expressed that plants are injured by salt because of the high osmotic pressure in soil solutions and the consequent limitation to water uptake.   Such a view fails to take account of the salt uptake by the plant and the tendency here shown toward the establishment of an equilibrium between the plant and its substrate; but one must not lose sight of the fact that in some of these cases there is a loss of differential with the addition of salt, which suggests that at higher concentrations water uptake

TABLE 8 —*Freezing-point depression of expressed sap of various plants and of the corresponding culture solutions*

FREEZING-POINT DEPRESSION

Source	Date	Portion of plant	Control	Chloride		Sulfate		
				50 milli-equiva-lents	150 milli-equiva-lents	50 milli-equiva-lents	150 milli-equiva-lents	250 milli-equiva-lents
			° C.	° C.	° C	° C	° C	° C.
Culture solution			0 06	0 21	0 50	0 15	0 29	0 42
Milo	July 16	6th leaf from base of each plant.	.92	1 11	1 49	1.04	1 10	1 15
Alfalfa	(¹)	Entire stems and leaves.	1 14	1.26	1 37	1.15	1 25	1 40
Cotton	Nov. 6	Mature and sound main-stalk leaves.	1 15	1 19	1.31	1.15	1 16	1.23
Tomato	Sept 9	Mature leaflets	79	.90	1 18	89	1 01	1 14
Barley	July 5	Entire plants at thick-dough stage.	83	1.24	1.72	1.50	1.39	1 57
Sugar beet	Oct. 23	Sound mature leaf blades.	1 13	1.36	1.75	1 31	1 41	1 40

OSMOTIC DIFFERENTIALS BETWEEN SAP AND SOLUTION

				Atm.	Atm.	Atm	Atm.	Atm.	Atm.
Milo				10.3	10 8	11 1	10.7	10.4	8 8
Alfalfa				13 0	12 6	10 4	12.1	11 5	11.8
Cotton				13.1	11.8	9.7	12 1	10 4	.97
Tomato				8 8	8 3	8 2	8 9	8.6	8 6
Barley				9.2	12.4	14.7	16 2	13 2	13.8
Sugar beet				12.8	13.8	15 0	13 9	13 4	11.8
Average				11.2	11.6	11.5	12.3	11 2	10.8

¹ Average of 3 crops harvested July 9, Aug. 21, and Oct. 10.

might be appreciably slowed down provided the plants were otherwise able to tolerate the accumulated ions. No differential of less than 8 atomospheres, however, was found between the osmotic pressure of the expressed sap and the supporting culture solution in this experiment. Freezing-point depressions in degrees centigrade are related to osmotic pressures in atmospheres by the factor $12.06\Delta - 0.02\Delta^2$. Data of like character were found in experiments with wheat and Australian saltbush (7) grown on soils to which sodium chloride was added in successively larger amounts.

Although experimental data as specific as the foregoing do not appear elsewhere in the literature, it has frequently been stated that plants on saline soils have a higher salt concentration in their tissues than those on less saline soils. Such relations have been pointed to by Maximov (22) as a basis for his comment that the concept of physiological dryness of saline soils is to be regarded in a relative rather than an absolute sense. The water relations of plants in equilibrium with a saline substrate must, in the writer's opinion, be carefully differentiated from the wilting that is produced when salt or sugar is suddenly added to soils or solutions or when saline crusts are washed into the root zone by a shower. Wilting, in the few instances in which it was observed in these experiments, was always as pronounced in the control bed as in any of the salt beds.

WATER REQUIREMENTS

The water requirements of the combined plants in each of the beds are reported in table 9. Decreased water requirements were found for the 50-chloride and 50-sulfate beds, and water requirements higher than those of the controls were found for the 150-chloride and 250-sulfate beds. Increased sap concentrations are conducive to reduced transpiration rates (7), but because of the reduction in growth caused by salt, exposure to light and wind was increased, and this resulted in increased transpiration; transpiration in this experiment was thus influenced by two oppositely directed factors. Growth, measured as dry matter produced, enters into the water-requirement calculation as the denominator of the ratio in which transpiration is the numerator, and the salt and water relations as measured by water requirements are accordingly somewhat complicated.

TABLE 9.—*Water requirements of eight crops grown together in sand beds*

Nutrient solution	Total dry weight of plants	Total water lost [1]	Water per gram of dry material	Nutrient solution	Total dry weight of plants	Total water lost [1]	Water per gram of dry material
	Grams	*Liters*	*Grams*		*Grams*	*Liters*	*Grams*
Control	11,472	6,434	561	50-sulfate	8,910	4,785	537
50-chloride	8,724	4,511	517	150-sulfate	6,270	3,513	560
150-chloride	3,677	2,375	646	250-sulfate	3,658	2,377	650

[1] Includes both transpiration by the plants and evaporation from the beds.

BLOSSOM-END ROT OF TOMATOES

The subject of blossom-end rot of tomatoes is somewhat irrelevant to the principal topic of this paper, but, as shown in table 3, this disorder, absent from the control bed, became a factor of importance

in plant growth in the chloride and sulfate beds, particularly in the latter. In the 250-sulfate bed, 84 percent of the fruit was affected. The related data on the incidence of blossom-end rot and the composition of the expressed sap have been brought together in table 10.

TABLE 10.—*Incidence of blossom-end rot of tomatoes (grown in sand beds) in relation to calcium accumulation in leaf sap*

Nutrient solution	Fruits affected with blossom-end rot	Electrolytes in leaf sap (milliequivalents per liter)						Freezing-point depression	
		Calcium	Magnesium	Sodium	Potassium	Chloride	Sulfate	Sap from mature leaves	Differentials between solutions and mature leaves
	Percent							°C.	°C.
Control	0	115	83	6	161	25	150	0.79	0.73
50-chloride	7	164	130	11	129	71	152	.90	.69
150-chloride	34	205	130	14	154	155	128	1.18	.68
50-sulfate	2	89	111	15	177	23	177	.89	.74
150-sulfate	78	315	68	17	165	17	222	1.01	.72
250-sulfate	84	478	63	28	153	13	299	1.14	.72

Blossom-end rot of tomatoes has been found to develop under diverse cultural conditions, and it has been associated with a number of environmental factors; Robbins (*28*) has recently reviewed a part of the literature on this disease. From his own work with plants grown on nutrient solutions of varied concentration, he concluded that unfavorable osmotic pressure in fruit relative to that in vegetative shoots, causing water withdrawal and tissue break-down, could be assigned as a cause.

On the basis of the observations made possible by the present experiments, it appears that neither unfavorable osmotic relations between the plants and solutions nor the accumulation of potassium or sodium can be assigned as a direct cause of the blossom-end rot induced by the treatments. Although the possibility that high chloride and high sulfate were independent causes cannot be entirely eliminated, the data suggest that calcium and magnesium accumulation singly or combined were important contributing factors.

The reasoning, applicable at least to this experiment, that seems to cast doubt on high osmotic pressure in the leaves and consequent unfavorable water relations in fruits as a cause of blossom-end rot, has to do in part with the fact that only 34 percent of the fruits were affected in the high-chloride bed, where the freezing-point depression was 1.18° C. in the expressed leaf sap, whereas in the 150-sulfate bed there was 78 percent of rot and a freezing-point depression of only 1.01°. As shown in table 10, there was little difference in the osmotic differentials between culture solutions and the leaves of tomatoes in any of the beds. Wilting of the tomatoes was not observed in any of the beds, but if incipient wilting did occur at any time as a result of insufficient moisture the chances for its occurrence would be greater in the control bed, from which there was greater water loss and in which there was no blossom-end rot.

For the leaves to withdraw water from the fruits it would be necessary to assume a water deficit in the leaves since, irrespective of os-

motic differentials, a turgid cell is limited in its expansion and water uptake by the cell wall which bounds the protoplast. In other words, it would not be reasonable to assume that a significant amount of water could be withdrawn from fruits by turgid leaf cells.

PLANT INJURY IN RELATION TO SALT ACCUMULATION

As shown in table 3, the addition of chloride and sulfate salts to the base nutrient resulted in a depression in the growth of nearly all plants and, as shown in tables 5 and 6, in the accumulation of chloride and sulfate in the sap in excess of the concentrations found in the control plants. With these increases in the concentration of chloride and sulfate in the plants there were also important changes in the concentration of the bases. As an aid to the reexamination of these data for causal relationships, the most pertinent of the material in these tables has been reassembled in table 11.

TABLE 11.—*Growth reductions and corresponding concentrations of chloride, sulfate, sodium, and total cations in expressed sap of six plants grown in outdoor sand beds with chloride and sulfate salts*

Crop plant and nutrient solution	Growth reduction (percentage of entire plant)	Expressed sap				pH
		Accumulation (milliequivalents per liter) of—				
		Chloride	Sulfate	Sodium	Total cations	
Milo:						
Control	0	26	21	1	316	4 72
50-chloride	37	81		4	399	4 63
150-chloride	85	267		14	555	4 38
50-sulfate	19			5	342	4.68
150-sulfate	57		45	9	426	4 57
250-sulfate	78		110	13	474	4 56
Alfalfa (third cutting):						
Control	0	25	38	25	459	[1] 5.31
50-chloride	11	79		39	472	[1] 5 25
150-chloride	51	171		65	526	[1] 5 13
50-sulfate	20		45	53	468	[1] 5 28
150-sulfate	25		67	81	482	[1] 5.25
250-sulfate	43		118	121	550	[1] 5 32
Cotton:						
Control	0	18	183	18	605	
50-chloride	26	76		26	680	
150-chloride	59	178		27	751	
50-sulfate	38		328	25	779	
150-sulfate	40		392	38	821	
250-sulfate	69		439	51	908	
Tomato:						
Control	0	25	150	6	365	
50-chloride	22	71		11	434	
150-chloride	80	155		14	503	
50-sulfate	23		177	15	392	
150-sulfate	49		222	17	565	
250-sulfate	72		299	28	722	
Sugar beet (fresh roots):						
Control	0	44	40	229	497	5.97
50-chloride	2	91		297	634	5.95
150-chloride	13	175		392	746	5 96
50-sulfate	22		55	258	551	5 93
150-sulfate	18		82	370	620	5 95
250-sulfate	41		110	347	609	5.99
Barley:						
Control		63				6.01
50-chloride		168				5.99
150-chloride		347				5.66
50-sulfate			89			6 00
150-sulfate			96			6.02
250-sulfate			91			6.02

[1] First cutting.

The method used for sap extraction does not permit of any orientation of the findings with respect to the more detailed loci of accumulation.   It seems safe to conclude, nevertheless, that salt accumulation extended both to the vacuolar and the cytoplasmic fluids, and accompanying the resulting equilibrium between these, the bases particularly may have become important constituents of the cell-wall materials bringing about structural changes or differences in extensibility.   These questions must wait upon further investigations, as must questions that have to do with the influence of these inorganic additions to the sap upon the elaboration, destruction, or action of plant hormones, or upon carbon assimilation and photosynthesis.

The reduction in pH values associated with chloride accumulation in the expressed sap must also for the time be regarded as having an uncertain significance.   A suggestion that importance may be attached to the change in pH values accompanying chloride accumulation is afforded by the fact that sugar beets accumulated chloride without change in hydrogen-ion concentration, and this plant was the only one measured which was not injured significantly by additions of 150 milliequivalents to the nutrient solution.   Van Itallie (*34*), observing a decrease in the pH values of tissues of potatoes grown on soils to which sodium chloride was added, concluded that this was the cause of chloride injury rather than the chloride itself.   He thought that the pH values were reduced because the plant took up more chloride than sodium.   Without questioning the importance of the buffer system of the plant and the dependence of normal activity on the maintenance of characteristic hydrogen-ion concentrations, it is nevertheless appropriate to leave open for further investigation the significance of change in the hydrogen-ion concentration and its meaning in terms of salt injury.   Furthermore, it is to be remembered that sulfate salts were likewise toxic and that large quantities of sulfur were found in the expressed saps and yet there was no associated change in the hydrogen-ion concentration.

Masaewa (*21*), working principally with buckwheat, advanced the idea that the physiological basis of injury is not the chloride itself but rather the fact that the excess chloride was taken up with calcium and that the normal K/Ca balance was thereby upset.   With the plants of the present experiments it might be difficult to substantiate a conclusion that potassium was deficient or that the K/Ca ratios were unfavorable to growth.   Along wholly different lines, Baslavskaja (*2*) reported lower chlorophyll content of potatoes and also higher water content in the leaves of both tobacco and potatoes as a result of the addition of chloride.   In potato leaves, a reduction in total carbohydrates (*3*) was associated with a reduction in chlorophyll.

EFFECT OF CHLORIDE AND SULFATE SALTS ON CORN, TOMATOES, AND WHEAT IN GREENHOUSE WATER-CULTURE EXPERIMENTS

Experiments in the greenhouse on corn, tomatoes, and wheat in water culture had three principal objectives, namely, to determine (1) whether the high toxicity of chloride and sulfate salts noted in the outdoor sand-culture experiments was in some way peculiar to the experimental methods employed, (2) the effects on growth of relatively low concentrations of chloride and sulfate salts, and (3) the character of the growth-depression curves through low as well as high

concentrations of these salts. For this reexamination of the problem wholly different methods were employed. A series of five water-culture experiments were conducted in the greenhouse; corn and tomatoes were used as test plants in both chloride and sulfate experiments; and finally there was an experiment with wheat in sodium chloride solutions.

METHODS AND MATERIALS

Though the aeration of water cultures is regarded as highly desirable, facilities could not be arranged for aerating the large number of small cultures essential for the purposes of the tests. To provide for some exchange of gas between the solution and the outside atmosphere, special covers were built for the quart mason jars used as culture vessels. Pieces of ¼-inch galvanized screen wire were cut in disks of such size that they would be held in place on top of the wide-mouth jars by the standard open-center screw rings such as are used for canning. An upright collar of galvanized iron, 1½ inches tall, was soldered to the screen wire (see figs. 5 and 7). After the selected seedlings from the germination trays had been placed on the screen, with their roots in the culture solution, beach pebbles were filled in around the stems to hold the plants upright. Thin paper squares slipped around the stems kept the tomato seedlings from sliding through the meshes of the screens.

The lighting conditions in the greenhouse in which the experiments were conducted were not very uniform. To provide uniform conditions for all cultures of a test, the jars were arranged in a single row near the outer edge of a clinostat the diameter of which was 6.2 feet. The circumference of the clinostat limited the number of cultures to about 50. The table made 1 revolution in 4 minutes.

The corn plants were the F_1 generation of a hybrid between two strains of a soft American Indian type known as Sacaton June. The parents of this seed [5] had each been selfed through six generations. Stone tomatoes and Baart wheat were used as the other test plants. All of the seeds were germinated on cotton netting over tap water (15) and, as soon as the roots were sufficiently developed to permit, transferred to the culture solutions. The cultures were left in the laboratory for 24 hours before they were placed in the lighted greenhouse.

The ionic concentrations of the base nutrient solutions used for these tests are shown in table 12. In the same table are shown the

TABLE 12.—*Composition of the base nutrient solutions used in the water culture greenhouse experiments reported in tables 14 to 18*

Crop and ion	Constituent of base nutrient (milliequivalents per liter)									Chloride and sulfate salts added (percent, as milliequivalents, of)—		
	Ca	Mg	Na	K	NH₄	NO₃	H₂PO₄	SO₄	Cl	Ca	Mg	Na
Corn (chloride)	8	4		4.25	4	12	0 25	8		25	25	50
Corn (sulfate)	8	4		4.25	4	12	.25	1	7	15	35	50
Tomato (chloride)	8	4		4.50	4	12	.50	8		25	25	50
Tomato (sulfate)	8	5	4	4 50	2	12	.50	1	10	15	35	50
Wheat (chloride)	14.5	3.6		7.20		17 7	4.00	3.6				100

[5] Supplied by J. H. Kempton and the late G. N. Collins, of the Bureau of Plant Industry, U. S. Department of Agriculture.

proportions of calcium, magnesium, and sodium salts used in the intro-
duction of the additional chloride and sulfate. In the four experi-
ments with corn and tomatoes, 50 percent of the chloride and sulfate
was introduced as the sodium salt. The remaining 50 percent was
added as calcium and magnesium salts. The culture solutions were
prepared from chemicals of reagent or equal quality. There was also
added to the solutions 1 p. p. m. of boron, 0.1 p. p. m. of zinc, and
0.1 p. p. m. of manganese, and, in very small amounts, a mixture of
28 additional elements. Iron citrate was added to the newly prepared
solutions to give a concentration of 5 p. p. m. of iron, and more was
added occasionally thereafter as required. In the experiment with
wheat, which was designed to parallel the experiments of Lipman,
Davis, and West (*17*), chloride was added as the sodium salt and the
base nutrient solution contained the same proportions of nutritive
ions that they used. The concentrations of the chloride and sulfate
employed in the successive experiments are shown in conjunction
with the results reported in tables 14 to 18. Dates, duration of the
experiments, and changes of solutions are recorded in table 13.

TABLE 13.—*Duration of and changes of nutrient solutions in the water-culture
greenhouse experiments reported in tables 14 to 18*

Crop and ion	Test started	Test ended	Dura-tion	Period from start to change of nutrient solution			
			Days	Days	Days	Days	Days
	1936	*1936*					
Corn (chloride)	Apr 4	May 2	28	12	21	26	
Corn (sulfate)	June 25	July 21	26	14	20		
Tomato (chloride)	Oct 16	Nov. 20	35	14	20		
	1937	*1937*					
Tomato (sulfate)	Jan 8	Feb 19	42	5	25	32	38
Wheat (sodium chloride)	Mar 21	May 10	50	44			

Transpiration data were obtained by measuring the volume of water
added to the cultures to replace that taken up by the plants. A check
jar without plants was carried through each experiment, and the
evaporation from it was deducted from the total water loss from each
of the cultures.

It should be pointed out that the results of the five experiments are
not wholly comparable with one another, in terms of the degree of salt
toxicity or the relative toxicity of chloride and sulfate salts, because
of the differences in the greenhouse temperatures, day lengths, and
other factors related to the season of the year in which the successive
experiments were conducted, as well as differences in maturity of the
plants and the composition of the base nutrient solutions.

CORN IN CHLORIDE SOLUTIONS (EXPERIMENT 3)

As shown in table 14 and figure 4, the growth of corn in this experi-
ment was depressed by relatively low concentrations of chloride.
Just where injury first occurred is difficult to determine, because of
the reaction of 2 of the 12 plants in 8 milliequivalents of chloride;
these 2 plants had green weights of 19.8 and 24.5 gm., whereas the
average of the weights of the 8 plants in the other 2 jars of the treat-
ment was 12.7 gm., suggesting that the 2 plants were in some way dif-
ferent and that there would be justification for disregarding either this

TABLE 14.— *Effect of chloride salts on growth of corn in water cultures (experiment 3)*

Chloride concentration (m. e./l.)	Jars	Plants	Average green weight of tops per plant	Standard error	Average dry weight of tops per plant	Average dry weight of entire plants	Moisture in green tops	Roots [1]	Water requirement [2]
	Number	*Number*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Grams*
(3)	10	40	14 8	0 41	1 13	1.54	92	26.9	254
2	10	40	14 9	.38	1 11	1.55	93	28.4	246
4	3	12	14.7	.47	1 08	1.54	93	29.6	242
6	3	12	13 8	71	1 01	1.41	93	28.4	252
8	3	12	15.0	1.08	1.10	1.52	93	27.4	256
10	3	12	12 7	.55	.92	1 28	93	27 9	259
15	3	12	12.3	.64	.92	1 28	93	28.2	264
20	3	12	11.1	.92	.81	1 15	93	29.7	268
35	3	12	9 9	.44	79	1.16	92	32 3	236
50	3	12	9.4	.74	.78	1.22	92	35.8	222
75	3	12	6.5	.52	.53	.88	92	39 4	244
100	3	12	6.4	.44	.58	.96	91	41.1	179

[1] Measured as percentage of entire plants.
[2] Measured as grams of water per gram of dry material.
[3] Trace.
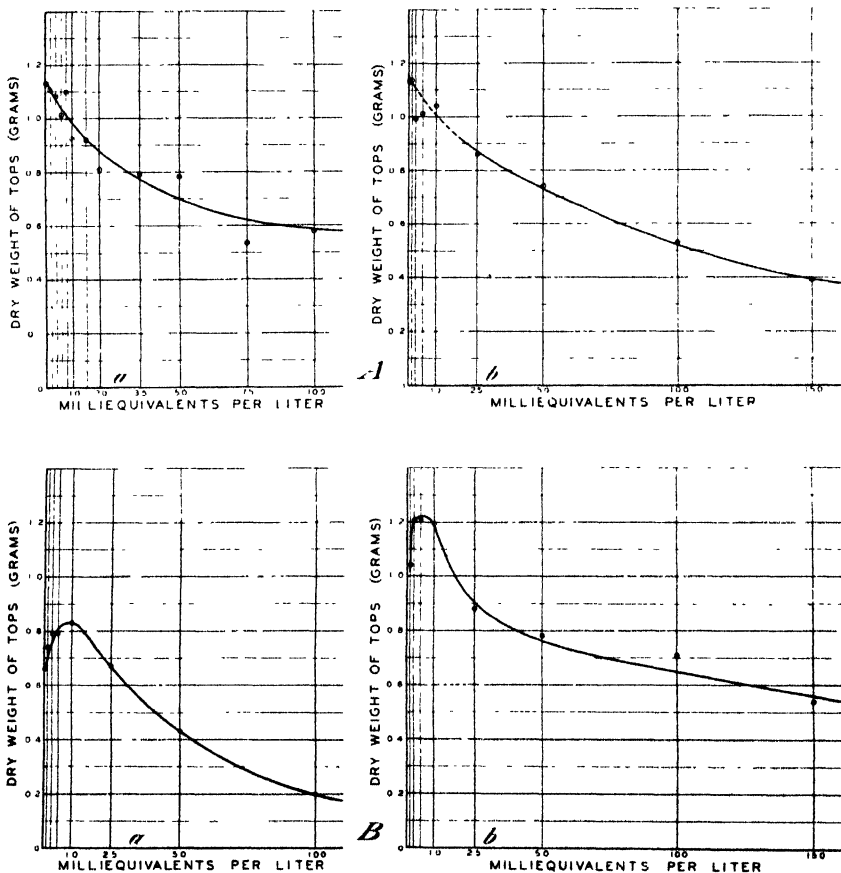
FIGURE 4.—Depression in growth of plants in greenhouse water-culture experiments; *A*, Corn with chloride (*a*) and sulfate (*b*) salts; *B*, tomatoes with chloride (*a*) and sulfate (*b*) salts.   (Experiments 3, 4, 5, 6, respectively.)

jar or the entire treatment. If this is done, a decreasing growth trend follows from the lowest to the highest concentrations.

There was little effect of the treatments on the moisture content of the plants other than that associated with the burning of the tips of leaves in the higher concentrations. In these higher concentrations the weight of roots relative to the weight of tops increased and the water-requirement values decreased.

The toxicity of chloride per unit of salt added being greater over the range from 4 to 20 milliequivalents than from 20 to 100, the growth-depression curve (fig. 4) tended to flatten with increasing concentrations of salts.

<div align="center">CORN IN SULFATE SOLUTIONS (EXPERIMENT 4)</div>

A sulfate concentration of 1 milliequivalent per liter resulted in slightly larger plants than 2.5 milliequivalents, indicating that under the conditions of experiment 4 the sulfate requirements of corn were relatively low (table 15). As in the preceding test with corn, there were decreasing moisture percentages in the green plants as the amount of tip burning increased. The weight of roots relative to entire plants increased, and the water requirements decreased through the higher sulfate concentrations. These relations being the same as in the preceding experiment with corn in chloride solutions, the effect cannot be regarded as peculiar to either of these anions. The growth-depression curve (fig. 4, A, b) resembles that for corn in chloride (fig. 4, A, a).

TABLE 15.—*Effect of sulfate salts on growth of corn in water cultures (experiment 4)*

Sulfate concentration (m. e /l)	Jars	Plants	Average green weight of tops per plant	Stand-ard error	Average dry weight of tops per plant	Average dry weight of entire plants	Mois-ture in green tops	Roots [1]	Water require-ment [2]
	Number	Number	Grams	Gram	Grams	Grams	Percent	Percent	Grams
1	6	24	13 0	0 73	1 13	1 51	91	25 0	414
2 5	6	24	10 7	56	99	1 33	91	25 4	416
5	6	24	11 0	66	1 01	1 34	91	24 9	420
10	6	24	11 9	78	1 04	1 38	91	24 3	418
25	6	24	8. 8	40	86	1 20	90	28 1	401
50	6	24	7 6	.46	74	1 05	90	29 2	370
100	6	24	4 0	40	53	82	87	35 0	289
150	6	24	2 3	.27	.39	.62	83	36 8	228

[1] Measured as percentage of entire plants
[2] Measured as grams of water per gram of dry material.

<div align="center">TOMATOES IN CHLORIDE SOLUTIONS (EXPERIMENT 5)</div>

Evidence of a substantial benefit to tomatoes from chloride concentration as high as 10 milliequivalents per liter resulted from experiment 5 (table 16). This evidence of a beneficial effect of chloride ion on tomatoes finds confirmation in a subsequent experiment reported in this paper (p. 388). No deficiency symptoms could be observed at concentrations below 10 milliequivalents nor did the leaves of the plants burn or show other symptoms of diagnostic value at the high chloride concentrations.

Chloride was without effect on the moisture content of the green tops. The lowest percentage of roots was found in the range of concentrations that produced the greatest weight of entire plants. The water-requirement values decreased through the series of concentra-

tions. Analysis of the leaves of these tomatoes, grown in unaerated water cultures, showed relatively more chloride than was found in tomato leaves from the outdoor sand-culture experiment.

TABLE 16.—*Effect of chloride salts on growth of tomatoes in water cultures (experiment 5)*

Chloride concentration (m. e./l)	Jars	Plants	Average green weight of tops per plant	Standard error	Average dry weight of tops per plant	Average dry weight of entire plants	Moisture in green tops	Roots [1]	Water requirement [2]
	Number	Number	Grams	Gram	Gram	Grams	Percent	Percent	Grams
(3)	6	18	9 1	0 28	0. 66	0. 88	93	24. 1	562
1	6	18	11 4	.36	74	.95	94	21 6	537
2.5	6	18	12 1	. 42	79	1 01	93	21 6	524
5	6	18	12 7	. 44	. 79	98	94	18. 7	540
10	6	18	13. 6	. 49	83	1 03	94	20 7	527
25	6	18	10 6	55	. 67	86	94	22 6	479
50	6	18	6 5	. 40	43	57	93	24 5	452
100	6	18	3. 0	. 19	20	29	93	31 2	437

[1] Measured as percentage of entire plants
[2] Measured as grams of water per gram of dry material
[3] Trace

TOMATOES IN SULFATE SOLUTIONS (EXPERIMENT 6)

In sulfate concentrations of 2.5, 5.0, and 10 milliequivalents the tomato plants were of approximately equal weight. Growth was slightly depressed in the solutions with only 1 milliequivalent of sulfate, and in solutions with more than 10 milliequivalents of sulfate successive reductions in growth occurred. The moisture content of the plants decreased slightly and the water-requirement values decreased considerably as the concentration of sulfate was increased (table 17). Symptoms of injury other than decrease in size were not in evidence. Cultures from this experiment are shown in figure 5.

TABLE 17.—*Effect of sulfate salts on growth of tomatoes in water cultures (experiment 6)*

Sulfate concentration (m. e /l)	Jars	Plants	Average green weight of tops per plant	Standard error	Average dry weight of tops per plant	Average dry weight of entire plants	Moisture in green tops	Roots [1]	Water requirement [2]
	Number	Number	Grams	Grams	Grams	Grams	Percent	Percent	Grams
1	6	18	16 2	0 65	1 04	1 27	94	18 2	524
2 5	6	18	19 5	1 05	1 21	1 45	94	16 4	514
5	6	18	19 6	1 03	1 21	1 43	94	15 5	519
10	6	18	18 4	. 97	1 19	1.44	94	17 6	494
25	6	18	12. 7	. 96	. 88	1.08	93	19 0	497
50	6	18	10. 8	. 67	. 78	. 99	93	20 7	449
100	6	18	9 2	. 66	. 71	. 88	92	19. 0	388
150	6	18	6 3	. 29	54	. 67	91	20 1	365

[1] Measured as percentage of entire plants
[2] Measured as grams of water per gram of dry material.

WHEAT IN SODIUM CHLORIDE SOLUTIONS (EXPERIMENT 7)

In the experiment with wheat grown in chloride solutions, boron, manganese, and zinc, together with a very small amount of a mixture of 28 additional elements, were added to four of the eight cultures of
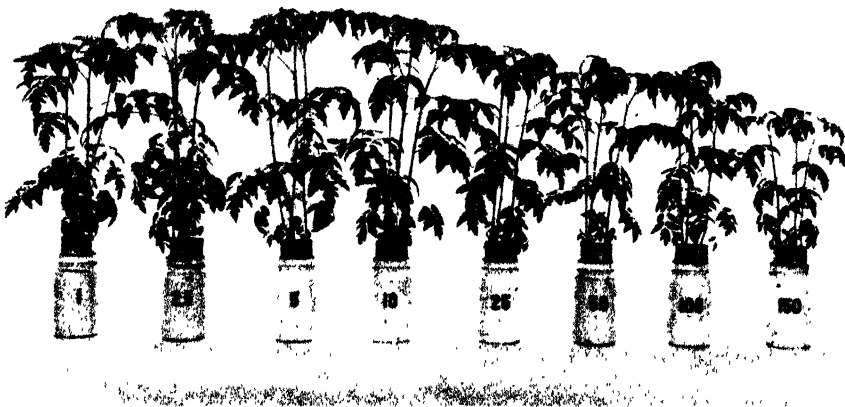
FIGURE 5.—Cultures of tomatoes grown in solutions with sulfate.   Figures on jars show concentrations of sulfate ion in milliequivalents per liter.   (Experiment 6)

each treatment and omitted from the other four.   The plants that received the added elements made only slightly better growth than those that did not (table 18).   The graphs (fig. 6) of the two sets of plants are otherwise similar.   Both indicate that 10 milliequivalents

TABLE 18.   *Effect of sodium chloride on growth of wheat in water cultures (experiment 7)*

[Intended to reproduce an experiment by Lipman, Davis, and West (17)]

WITH TRACE ELEMENTS B, MN, ZN, AND 28 OTHERS

NaCl concentration (m e /l.)	Green weight of 6 plants per jar					Average dry weight of entire plants per jar	Moisture in entire green plants	Roots[1]	Water requirement[2]
	Jar 1	Jar 2	Jar 3	Jar 4	Average				
	Grams	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Percent*	*Percent*	*Grams*
(1)	71	75	72	66	71 0	7 48	89	21. 1	648
10	66	69	77	81	73 3	7 23	90	23 2	702
50	34	42	40	36	38 0	4 80	87	28 1	612
100	14	8	12	18	13 0	2 05	84	18 2	525
200						4 47	---	---	545
300						(5)	-		----

WITHOUT TRACE ELEMENTS

(3)	71	75	74	58	69 5	7 10	90	21 4	699
10	76	72	62	64	68 5	6. 73	90	23 4	694
50	34	39	30	33	34 0	4 37	87	25 3	651
100	10	12	12	12	11 5	1 93	83	19. 4	557
200						6 43	---		570
300						(5)			----

[1] Measured as percentage of entire plants.
[2] Measured as grams of water per gram of dry material.
[3] Trace.
[4] Only 2 plants survived.
[5] All plants died at an early stage.
[6] Only 3 plants survived.

of sodium chloride was slightly toxic and 50 and 100 milliequivalents decidedly so. Plants in 200 milliequivalents made little growth.

The methods employed in this test were not different in essential details from those employed by Lipman, Davis, and West (17) in their work with wheat in sodium chloride solutions. They found that depressed growth resulted only when the concentration of chloride was increased to 90 milliequivalents per liter. In their work, conducted as it was in the years 1920 to 1922, elements such as boron, manganese, and zinc were not added to the solutions except when introduced as impurities. The present experiment does not provide an explanation of the differences in the two sets of results. It is possible, however, that their base nutrient salts were exceptionally free from trace elements and that these elements were introduced with the sodium chloride in amounts just sufficient through the successive concentrations up to 90 milliequivalents to offset salt toxicity.
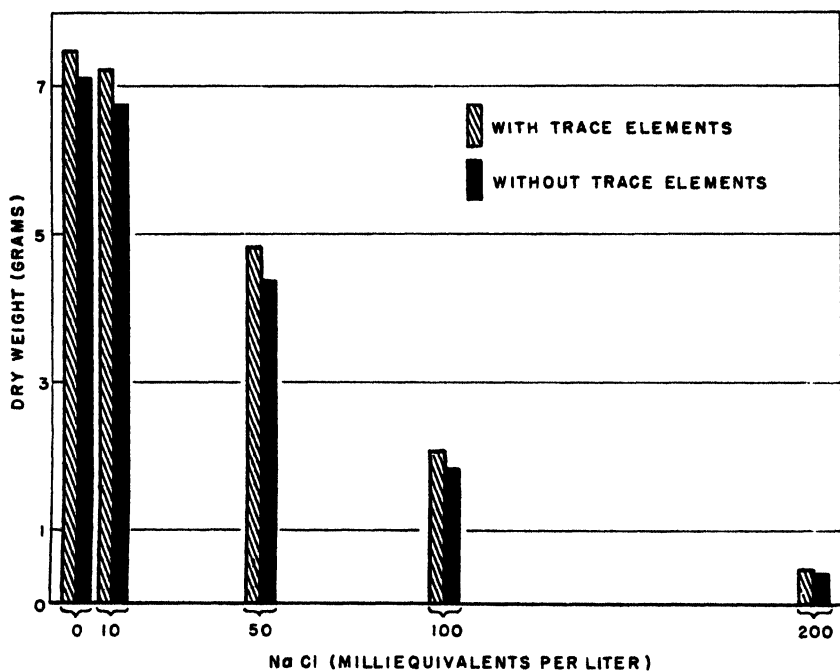
FIGURE 6.—Effect of sodium chloride on growth of wheat with and without the addition of trace elements.

The moisture content of the green plants (table 18) decreased with increased burning. Water requirements were reduced with the addition of salt as in the preceding experiments with corn and cotton and as in the Lipman, Davis, and West (17) experiments. It may be observed (fig. 7), that 10 milliequivalents of chloride tended to increase the height but not the weight of wheat plants.

FIGURE 7.—Wheat grown in solutions with sodium chloride. Numbers on jars show concentrations of sodium chloride in milliequivalents per liter. (Experiment 7.)

BENEFICIAL EFFECT OF CHLORIDE ON THE EARLY GROWTH OF TOMATO AND COTTON PLANTS (EXPERIMENT 8)

Following the observation of the beneficial effects of a small quantity of chloride ion on tomatoes grown in the water-culture tests, two somewhat incidental experiments were conducted in sand cultures in the greenhouse for the purpose of further observation.

Tomatoes were grown in 27 sand cultures supplied with only the trace of chloride originating as an impurity in the sand and nutrient solution and in 27 otherwise similar cultures maintained with a nutrient solution containing 3 milliequivalents of chloride. After the tomato plants had been cropped the experiment was repeated with cotton. Both experiments are reported in table 19. The base nutrient solution used for the tomatoes contained, in addition to the trace elements boron, manganese, and zinc, 4 millimoles each of calcium nitrate ($Ca(NO_3)_2$) and potassium nitrate (KNO_3), 2 millimoles per liter each of magnesium sulfate ($MgSO_4$) and ammonium sulfate ($(NH_4)_2SO_4$), and 1 millimole of monopotassium phosphate (KH_2PO_4). In the cotton test 0.5 millimole of sodium sulfate (Na_2SO_4) was added to the above solution, and half of the ammonium sulfate was omitted. The chloride ion was supplied as sodium chloride ($NaCl$).

The culture vessels were 2-quart Oldberg-type percolator tubes. These were fitted with a wad of glass wool above the tubular opening at the base and filled to within about 3 inches of the top with quartz sand. Two 1-quart mason jars were used in conjunction with each. One of these, placed beneath the tubes, collected percolate when nutrient solution was applied from the other. After the daily or more frequent passage of the nutrient solution through the sand it was

returned to volume with distilled water, and the positions of the two jars were reversed.

Eighteen of the percolator tubes were suspended through openings near the rim of each of three clinostats. These clinostats, which were 4.5 feet in diameter, were set flush with a 15-foot greenhouse bench and formed rotating disks in the top of it. The collecting jars rested at an appropriate height on circular shelves suspended from the tops of the rotating clinostats. The three clinostats were driven by a single endless belt.

In each culture the tomato plants were grown from July 27 to September 22, 1936, and the cotton plants from January 2 to March 10, 1937.

Both tomatoes and cotton responded to the addition of 3 milliequivalents of chloride. On the basis of clinostats A, B, and C, taken collectively, the growth of tomatoes as measured by green weight was greater by 35 percent, and the growth of cotton as measured by green weight was greater by 81 percent, when supplied with 3 milliequivalents of chloride than when only a trace was present (table 19).

TABLE 19.—*Effect of 3 milliequivalents of chloride on growth of tomatoes and cotton in sand cultures in the greenhouse (experiment 8)*

Crop plant and treatment	Clino-stat	Cultures	Plants	Average green weight per plant	Standard error
		Number	*Number*	*Grams*	*Grams*
Tomatoes:					
Trace of chloride	A	9	27	18.6	1 02
	B	9	27	14 9	.75
	C	9	27	15 9	.57
Total or average		27	81	16 5	49
3 milliequivalents of chloride	A	9	27	25 5	.95
	B	9	27	23 0	1 03
	C	9	27	18 1	.81
Total or average		27	81	22 2	64
Cotton:					
Trace of chloride	A	9	27	10 5	1 20
	B	9	27	11.7	1 41
	C	9	27	14 2	1 17
Total or average		27	81	12.1	.78
3 milliequivalents of chloride	A	9	27	28 1	1 91
	B	9	27	20 8	1.03
	C	9	27	16 7	1.21
Total or average		27	81	21.9	1 21

The differences in the growth of the similarly treated plants on the three adjacent clinostats is probably typical of what takes place in many small greenhouses. The distance between the centers of adjacent clinostats was less than 5 feet, and they were rotated continuously both night and day. The center line of the clinostat assembly was parallel with and 5.5 feet away from the south face of the greenhouse.

In the following discussion, references are made to the extensive literature that exists on the effects of relatively small amounts of chloride on the growth of plants. The results reported in tables 14 and 17 seem especially noteworthy in that in most of the earlier work much lower concentrations were customarily used and rarely have responses as great as these been obtained.

GENERAL DISCUSSION

All irrigation waters, whether diverted from streams or pumped from underground aquifers, carry salts in solution. These salts are variously derived from the rock and alluvial materials with which the waters have been in contact, from the residues of past or present lakes, marshes, inland seas, and estuaries, and from the drainage of upstream or overlying irrigated lands and the seepage from subirrigated areas supporting vegetation along watercourses. By reason of evaporation from soil surfaces, the uptake of water in excess of salts by plants, and the rise of salt from saline subsoils and water tables, it is quite regularly found that the soil solutions are more saline than the irrigation supply. Depending upon the extent of root-zone leaching and the removal of the percolate by drainage, the accumulation of salt in soils may be of nominal magnitude, or concentration may increase to such a degree that agricultural operations become unprofitable. The salt constituents of irrigation water may affect plant growth adversely owing to their excessive accumulation in the soil and later in the plants or to changes in the physical and chemical characteristics of soils that impede water penetration or otherwise cause the soil environment to be less favorable as a medium for root development.

Whatever the source or kind of salt, the extent of its accumulation, or the mechanism of its ill effects, salinity is apt to be an important factor in irrigation agriculture wherever it is practiced. In the discussion that follows, features of the salinity problem are briefly touched upon that have seemed to the writer to merit consideration not only from the standpoint of the data that have preceded but in a broader way from that of plant and salt relations under conditions of irrigation agriculture.

The absence of leaf symptoms or other pronounced outward abnormalities in a number of plants whose growth was depressed by salt has been noted in this paper. Before undertaking the present experiments the author had come to regard this feature of the salt relations of plants as one of special interest. Both in the field and in minor experiments, where comparisons were possible between plants growing on saline and those growing on nonsaline soils, it had been observed that many salt-injured plants developed no symptoms of diagnostic significance. These observations suggested that a substantial proportion of the curtailed production of crops in irrigated areas that was attributed to nutritional deficiencies or unfavorable water relations was in fact due to saline conditions customarily regarded as insufficiently high to be a cause of reduced yields.

In the absence of symptoms of injury and without an opportunity for direct growth comparisons with plants on adjacent nonsaline soils, the drawing of significant conclusions on salt injury under field conditions must be regarded as difficult. Loughridge (*19, p. 39*), for example, published a table under the heading "Highest amount of alkali in which fruit trees, other trees, and small cultures were found unaffected." This table, which has been reprinted in one of the important soil-reference books (*14*), has had an extensive influence, and yet it is based entirely upon the appearance of plants in the field as judged by its author.

The two 13-year-old grapefruit orchards shown in figures 8 and 9 are illustrative of the foregoing situation as it is encountered when

FIGURE 8.—Thirteen-year-old grapefruit grove on Superstition sand, Yuma Mesa, Ariz.; irrigated with Colorado River water. The yield in 1936 was 546 pounds of fruit per tree. The character of this soil and the irrigation practice have been such that there has been little accumulation of salt in the root zone, i. e., the water has been applied substantially in excess of evaporation and transpiration losses. The grove is vigorous and the trees large. Analyses of displaced soil solutions and of the irrigation water are shown in table 20.

one endeavors to interpret the significance of salt concentrations found in field soils. Both of these orchards were on deep alluvial soils and both were irrigated from the same water supply. The poor orchard on the relatively impermeable saline soil (fig. 9), notwithstanding the contrast with the good orchard (fig. 8), appeared reasonably healthy

FIGURE 9.—Thirteen-year-old grapefruit grove on Holtville silty clay, Meloland, Imperial Valley, Calif.; irrigated with Colorado River water. The yield is less than 100 pounds of fruit per tree. The character of the soil in this orchard is such that it apparently has not been possible to leach the salt residues of the irrigation water beyond the root zone. The trees have good color and are heavily foliated. There are few yellow or burned leaves. The trees and individual leaves are smaller than those of the trees shown in figure 8, but salt-injury symptoms of diagnostic value are otherwise lacking. Analyses of displaced soil solutions and of the irrigation water are shown in table 20.

and the foliage was of good color, but the yield was low. Several years later, when the poor orchard was again visited, deadwood was observed in the tops of some of the trees, the leaves were small and many were yellowed to varying degrees, with some burning of the leaf tips.

Not a little of the difficulty that has attended the interpretation of the analyses of field soils in terms of plant reactions has been due to the common practice of referencing the quantity of salt found to the dry weight of the soil instead of stating the concentration as of the soil solution. The critical nature of this consideration can be illustrated by assuming two soils- one with a moisture equivalent or field-carrying capacity of 4 percent and the other of 40 percent. Should each of these soils contain 500 p. p. m., or 0.05 percent, of chloride on dry-weight basis, then the soil-solution concentration at the moisture equivalent in the first soil would be 12,500 p. p. m. and in the second soil 1,250 p. p. m. A creditable growth of a crop like milo could be expected on the latter soil, but on the first few if any agricultural plants would survive. The analyses of the soil solution reported in table 20 are useful as a further illustration. In terms of soil solutions, the concentration of chloride found in the fourth foot of the poor orchard (fig. 9) is 9.5 times as high as that in the fourth foot of the good orchard. Had these same quantities of chloride been recorded in terms of the dry weight of soil, the concentration in the poor orchard would have been 95.6 times that in the good orchard.

TABLE 20.- *Analyses of displaced soil solutions of Superstition sand and Holtville silty clay, from grapefruit groves in Arizona and California, and of the irrigation water from the Colorado River as sampled at Yuma, Ariz.*

[Analyses of displaced soil solutions are adjusted to the moisture equivalent]

Origin and depth of sample	Mois-ture equiv-alent	pH	Conduct-ance (K × 10⁵ at 25° C)	Boron	Other constituents (milliequivalents per liter)							
					Ca	Mg	Na	K	HCO₃	SO₄	Cl	NO₃
Superstition sand [1]				*P p m*								
First foot	11 4	7 4	219	0.84	6.48	6 58	9.51	0 72	5 40	7 65	9.51	0.72
Second foot	3 4	6.4	226	.44	8 71	4 47	9.62	.80	3.89	9.62	8 66	.98
Third foot	4 6	6.7	200	.38	7.37	3.83	8 83	.60	3.25	9 11	7.24	.87
Fourth foot	5 4	7.0	210	.36	7.75	4.20	9 42	.53	2.10	11 27	7 25	90
Fifth foot	3 8	6.6	240	.59	8.18	4.73	11.06	.66	2 29	14.24	7 62	1 19
Sixth foot	3.9	6 6	320	.48	11.79	6.62	14.49	.92	1.75	19.43	12.21	1.22
Holtville silty clay [2]												
First foot	22 7	7 7	685	1.05	24.46	21.57	38.31	.95	4.07	54.37	27.15	.54
Second foot	18.1	7.7	1,087	1 03	37.40	28.57	69.10	.85	4.26	75.69	54 03	1 09
Third foot	25 9	7 4	1,480	.75	38.59	39.86	90.70	.22	1.99	54.37	119.32	.45
Fourth foot	45.7	7 3	1,229	.87	33.39	34.07	78.92	.15	1.51	39.81	106 75	.40
Fifth foot	45 3	7 0	1,092	1.25	26.33	28.37	76.18	.08	.90	47.96	85.21	.45
Sixth foot	37.4	7.6	769	.79	18.69	18 14	54.87	([3])	.85	48.68	45.65	.37
Colorado River [4]			137	.17	5.41	3.01	6 47		3 19	7.71	3.68	.11

[1] Soil samples collected June 30, 1936.   [2] Soil samples collected July 1, 1936.   [3] Trace.
[4] Eight-year, unweighted weekly average (Oct 1, 1928, to Sept. 30, 1936).

Inasmuch as the reaction of plants to salt constituents of the soil is dependent upon the concentration of the solution, there is obvious need for investigators to agree upon the most suitable moisture condition of the soil to be taken as a base. The moisture equivalent corresponds approximately to a constant value of the capillary potential or soil-moisture tension for different soils. With this base it is at once apparent that concentrations will tend to double as the

wilting coefficient of the soil is approached. Breazeale (4) has suggested referencing salt concentrations to the moisture content of soils at the wilting coefficient. However, the moisture equivalent of soils, which corresponds roughly in well-drained soils to field capacity, is more easily ascertained than the wilting coefficient, and where displacement methods are employed the soils are customarily wet to the moisture equivalent.

Plants have been grown extensively in undrained potted soils in which known amounts of salt were incorporated by careful mixing. Water applied to the surface of the soils to maintain the plants would unavoidably wash the salts downward, leaving the upper and better aerated zones relatively free from salt. In one investigation (24, 25), where 4-gallon stone jars were used in this manner, it was found that additions of sodium carbonate and sodium chloride as high as 0.4˙ percent of the dry weight of the soil and sodium sulfate as high as 0.9 percent were as often stimulating as toxic. This type of finding is not in accord with the results reported in this paper.

The water-holding capacity of suspended soils, that is, soils in containers 1 or 2 feet deep, even when provided with drainage, may exceed that of the same soil in the field two or three times, giving rise to the possibility of conditions of poor aeration such as exist in waterlogged lands. The conditions in poorly aerated soils are those conducive to the reduction of sulfate and nitrate into more toxic forms.

Experiments conducted with culture solutions may lead to erroneous conclusions if special care is not employed to maintain the concentrations of nutrient ions in the control cultures at levels as high as those in the salt cultures. The significance of this consideration was once brought out in an experiment with cotton plants grown in automatically flushed greenhouse sand cultures. Because of the pressure of other work, the writer was unable to change the nutrient solutions in the reservoirs as soon as planned; this being the case, it seemed best to give up the original purpose for which the plants were being grown and continue to care for them without further change of the solutions. At the time the solutions should have been replaced, the control plants were far ahead of the chloride and sulfate plants. The growth rate of the control plants soon declined, whereas that of the chloride and sulfate plants was maintained for a time, with the result that by the end of the fourth week there was little difference either in general appearance or size of the three lots. This experiment would seem to indicate that a plant such as cotton, with an indeterminate growth habit, can utilize a like supply of nutrients from a saline substrate about as effectively as a more rapidly growing plant on a nonsaline substrate, but it requires more time to do it.

It is not unlikely that conditions such as those outlined in the preceding paragraph may sometimes lead to the production of as high yields on saline soils of limited fertility as are obtained on otherwise similar nonsaline soils. In recognizing this as a possibility, however, account should be taken of the fact that salt concentrations typically, though not universally, increase with depth, as was illustrated in table 20. Experiments that can only be touched upon here have shown that, when the roots of a plant are divided between two vessels, the part of the root system in a concentrated solution does not develop as rapidly as the part in a dilute solution. In other words, to apply

the foregoing observation to the field, it seems necessary to recognize that the roots of the slow-growing crop on saline soil might never fully occupy the deeper and more saline portion of the soil and, failing in this respect, its total supply of nutrients would not be equal to that available to the crop on the nonsaline soil.

It has come to be generally recognized that with the introduction of salts, particularly sodium salts, into soils there may be marked effects upon the availability of nutritive ions. Effects of this kind on the growth of plants are apt to be outstanding in pot experiments since, in such case, the soil volume relative to the number of plants is customarily small and any increase in the availability of nutritive ions is commonly reflected by substantial increase in growth. Potassium released by base exchange processes usually receives the primary consideration in this connection, but the indirect effects are not necessarily limited to this ion. Sokoloff (*31*), for example, has found higher nitrate concentrations in soils treated with sodium salts than in soils treated with calcium salts. The increases in soluble nitrogen were accompanied by the evolution of carbon dioxide, and the effect is attributed by Sokoloff to a more rapid biological oxidation of the nitrogen of humus. It has been found by experiments not included in this report that better growth resulted on a low-nitrogen soil when sodium chloride was added than when calcium chloride was added; whereas with the addition of nitrogen the reverse was observed. Without the addition of nitrogen, the sodium treatment increased the growth of tomatoes and barley above that of the control plants; smaller increases were shown by cotton plants.

In some instances nitrate accumulation has been found to accompany chloride and sulfate accumulation in irrigated soils, and, if the concentration is not excessive, the productivity of the land may thereby be increased; but it does not follow that there is any substantial reduction in the toxicity of chloride or of sulfate if comparisons are made between saline and nonsaline soils at equal nitrate levels. In an experiment conducted during the winter and spring months in the outdoor sand beds, onions, alfalfa, barley, and table beets were grown in solutions containing 2 and 13 milliequivalents of nitrate ion in two control beds, in two beds with 100 milliequivalents of chloride, and in two beds with 200 milliequivalents of sulfate. Onions and alfalfa made in the order of 25 percent more growth in the low-nitrate than in the high-nitrate control; barley and beets showed an even greater advantage in the high-nitrate over the low-nitrate control. The yields of onions and alfalfa were nearly the same in the chloride and sulfate beds, and the barley yield was appreciably lower in the sulfate than in the chloride beds, but there was no clear evidence of a nitrate effect on the salt tolerance of any of these crops. The table beets, like the sugar beets of experiment 1, produced almost as well in 100 milliequivalents of chloride as in the control beds, and the nitrate advantage was retained. The injury of the table beets in the high-nitrate sulfate bed was substantial, corresponding to sulfate injury to sugar beets, and the nitrate advantage was marked; the yields of beets in the low- and high-nitrate control beds were respectively 100 and 176; in the chloride beds, 114 and 150; and in the sulfate beds, 71 and 141, all of the values being relative to the low-nitrate control. In each of the four crops the added nitrate depressed chloride accumulation in the sap and the

added chloride depressed nitrate accumulation. The addition of sulfate had little effect on either nitrate or chloride accumulation.

A somewhat general question has been raised in connection with the interpretation of some of the older sand- and water-culture studies. Many of these studies were conducted before the necessity for adding elements such as manganese, boron, and zinc to nutrient solutions was recognized, and there is therefore the possibility that essential trace elements may sometimes have been introduced as impurities with the chloride or sulfate salts and thus have improved the growth of the plants, offsetting the toxicity of the salts in the lower concentrations.

There is little information upon which to base conclusions as to the relationship between transpiration rates and salt accumulation in the plant and the consequent injury. Muenscher (*23*), in general agreement with numerous earlier investigators, who in nearly all instances confined their laboratory measurements to the determination of total ash, found that the ash of barley, expressed in percentage of total dry weight of whole plants, varied but slightly whether the plants were grown under conditions of high or low transpiration and regardless of how transpiration was reduced. Hoagland and Broyer,[6] on the other hand, found higher concentrations of sodium and chloride ions in the expressed sap of barley plants grown in a dry chamber than in that of plants grown in a humid chamber. They also reported that bromide ion was taken up from culture solutions and moved into the roots, stems, and leaves of squash and cotton more rapidly under the influence of light and humidity conditions conducive to high transpiration rates than under those conducive to low transpiration rates.

Ahi and Powers (*1*) have observed the toxicity of sea-water mixtures to saltgrass and of sodium chloride to alfalfa when these plants were grown in water cultures in cold and warm greenhouses. The effect of temperature on salt injury as shown by their data was not marked when growth relative to the controls in the respective greenhouses was computed in terms of percentage growth reductions. The investigations of Hoagland and Broyer (*15*) and of Prevot and Steward (*27*), dealing with other factors affecting accumulation, likewise have a bearing on the general subject of salt accumulation.

The results of experiments dealing with the long-standing question of the role of chloride as an essential or beneficial element and with the effects of chloride introduced into soils with fertilizers are to some extent in contrast with those that have dealt with the problem of salt accumulation and toxicity under conditions of irrigation agriculture. The investigational handicaps in these inquiries are similar, and as a consequence the findings have been diverse and often conflicting. The fact that plant species and varieties are commonly so clearly distinct from one another in their nutritional requirements and preferences, in their tolerances, and in their environmental responses has contributed in a major way to the difficulties of formulating generalizations. The yields of flax, buckwheat, and potatoes have been shown to be reduced and the quality of tobacco to be inferior, under some conditions, when potassium chloride rather than potassium sulfate has been used as a

fertilizer. On the other hand, a number of crop plants have been found to respond favorably to moderate amounts of chloride. Extensive reviews of this literature are provided by Tottingham (*32*) and Lomanitz (*18*) and more recently by Shestakov and Shvindenkov (*29, 30*) and by Masaewa (*21*). The findings of Garner et al. (*11*) on the reactions of tobacco to chloride are likewise pertinent.

Under the conditions that existed during the course of the outdoor sand-culture experiment, it was found that 100 milliequivalents of sulfate added as mixed sodium, calcium, and magnesium salts was about as toxic to dwarf milo, alfalfa, and cotton as 50 milliequivalents of chloride. Relations of this character have on several occasions suggested the possibility that the toxicity of chloride and sulfate might be related on the basis of equimolar concentrations or on the basis of equal electrical conductances or equal osmotic concentrations. It must be observed in this connection, however, that lemons withstood three or four times as many milliequivalents of sulfate as chloride, whereas beets withstood chloride better than sulfate. In other experiments it has been observed also that there are significant differences among plants in their reactions to both chloride and sulfate when these ions are added respectively as calcium, magnesium, and sodium salts; tomatoes, for example, even though accumulating little sodium (table 6), have been found to be substantially more tolerant to calcium chloride than to sodium chloride. In view of these diverse reactions and the specificity shown in ionic uptake by different plants, it seems improbable that chloride and sulfate toxicity can be evaluated on a simple physical or chemical basis.

SUMMARY

A series of crop plants was grown to maturity in each of six large outdoor sand cultures supplied with a base nutrient and with chloride and sulfate salts (50 percent as sodium) added; in milliequivalents per liter, as follows: Control, 50-chloride, 150-chloride, 50-sulfate, 150-sulfate, and 250-sulfate. The values for the growth of the plants in the 50-chloride bed and of the average of the plants in the 50- and 150-sulfate beds, measured as percentage of the controls, were respectively as follows: Lemon cuttings, 28 and 59; navy beans (seed), 39 and 45; dwarf milo (grain), 54 and 60; Chilean alfalfa (3 cuttings), 73 and 75; Acala cotton (seed cotton), 75 and 77; Stone tomatoes, 78 and 64; sugar beets (fresh roots), 98 and 80. Sulfate appeared to be about half as toxic as chloride to some plants, but the lemon was apparently four or more times as tolerant to sulfate as to chloride. Tomatoes and beets were more tolerant to 50 milliequivalents of chloride than to 100 milliequivalents of sulfate.

Barley, milo, and navy bean leaves were burned by chloride and sulfate salts, and occasional lemon leaves were burned. Alfalfa, cotton, tomato, and beet plants showed no burning of the leaves nor were there symptoms of diagnostic significance other than a reduction of leaf size in cotton and severe blossom-end rot of tomatoes.

The growth-depression curves showed no evidence of an abrupt point at which toxicity effects became pronounced. It was indicated instead that above some minimum concentration each successive unit of salt, if considered by itself, tended to produce a lesser depression in growth than the preceding unit.

The succulence of the crops, barley excepted, as measured by the quantity of sap expressed under standard conditions after freezing, was not influenced by the concentration of salt in the culture solutions.

Analyses of the expressed tissue fluids of the six crops examined showed much diversity in the proportions and quantities of ions accumulated in the sap. The ratios of concentration of salt constituents in the nutrient solution to the concentration in the sap of the control plants were high for nearly all ions. The base nutrient solution contained 0.6 milliequivalents of chloride, and the six crops growing in the control bed contained from 18 to 63 milliequivalents of chloride in their expressed sap. The addition of 50 to 150 milliequivalents of chloride to the base nutrient tended to produce a corresponding increase in the concentration of chloride in the expressed sap. Tenfold increases in the concentration of bases in the nutrient solutions by the addition of chloride salts and fourteenfold increases by the addition of sulfate salts in no case doubled the concentration of total bases in the expressed sap of any of these plants. Potassium accumulation was little affected by other ions. Cotton and tomatoes in all treatments had several times as much sulfur in their sap as the other plants. The hydrogen-ion concentration of the sap of a number of the plants was increased by chloride but not by sulfate.

On the basis of comparisons between electrical-conductivity measurements on the culture solutions and on the plant saps and the corresponding inorganic analyses, it is concluded that conductivity has little significance as a measure of the electrolyte content of expressed tissue fluids.

Increases in the osmotic pressure of expressed tissue fluids,[*] resulting from the salt additions, tended to parallel the increases in the osmotic pressure of the culture solutions. The data do not support the presumptive reasoning that has sometimes led to the view that plants on saline substrates are injured because of the high osmotic concentrations of the substrate and consequent limitations in the rate of water intake.

Less water was lost by transpiration and evaporation, per unit of dry matter produced, from the 50-chloride and 50-sulfate cultures than from the control bed. The water requirements of the 150-chloride and 250-sulfate plants exceeded those of the controls. The results point to the conclusion that the water requirements of plants growing on saline soils tend to be lower than those of plants on nonsaline soils but that this effect may be more than offset when the reduced growth causes an excessive exposure of plants to light and wind.

There was no blossom-end rot of tomatoes in the control bed, whereas 34 percent of the fruits in the 150-chloride bed and 78 percent in the 150-sulfate bed were affected. Unfavorable water relations due to high osmotic concentration in the solutions are discounted as a probable cause, and some relation to calcium and magnesium accumulation is indicated.

Four greenhouse experiments were conducted on corn and tomatoes grown in water cultures with mixed calcium, magnesium, and sodium chlorides and with mixed sulfate salts. Chloride and sulfate concentrations above a few milliequivalents were injurious to corn. Tomatoes showed a maximum growth with 10 milliequivalents of chloride and with 5 milliequivalents of sulfate. The growth-depression curves

of both plants in both chloride and sulfate salts tended to flatten out as the concentrations were increased, showing a higher degree of toxicity per unit of salt in the low concentrations than in the high concentrations. Similar results were obtained in an experiment with wheat in water cultures to which sodium chloride was added. Increasing the concentrations of chloride and sulfate salts throughout the higher concentration ranges reduced the water requirements and increased the weights of roots relative to the weights of the entire plants.

Confirmation of the evidence of a beneficial effect of low concentrations of chloride ion on the growth of tomatoes as observed in the water-culture experiments was obtained in sand cultures. Tomatoes and cotton made respectively 35 and 81 percent more growth on the basis of green weight with 3 milliequivalents of chloride (106.5 p. p. m.) in the nutrient solution than with a trace of chloride.

The terms "critical concentration," "limit of tolerance," and "threshold values," not infrequently appear in the salt-tolerance literature. These terms, which are borrowed from other biological fields, do not appear to be well suited to descriptions of the responses of plants to chloride and sulfate salts. There were no plant reactions or points on the injury curves in any of the experiments to which any of these terms were applicable. The limit of tolerance for any plant appears to be an intangible concept, since death takes place slowly over a range of concentrations, and on especially warm days plants growing in saline solutions may die rapidly.

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HEMICELLULOSES OF CORNSTALKS [1]

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INTRODUCTION

Although the hemicelluloses may be considered as one of the three major groups of constituents of such materials as cornstalks, cereal straws, hulls, cobs, hays, and similar agricultural materials, they have not been studied to a great extent.

Browne and Tollens (*2*) [2] hydrolyzed the pith of cornstalks with 6-percent sulfuric acid and identified *d*-xylose and *l*-arabinose in the hydrolysate. Also, by extracting the pith of cornstalks with warm 5-percent aqueous sodium hydroxide solution and then adding alcohol to the clarified alkaline extract, they obtained a crude hemicellulosic product that amounted to 17.4 percent of the weight of the pith. This product contained 4.16 percent of ash and 60.75 percent of pentosans and on hydrolysis with hydrochloric acid yielded *d*-xylose and *l*-arabinose.

Peterson and Hixon (*10*) digested cornstalks (previously extracted with a 1-percent aqueous ammonium hydroxide solution) with 5-percent aqueous sodium hydroxide solution at room temperature for 12 hours. The reaction mixture was filtered, and the digestion with 5-percent aqueous sodium hydroxide solution was repeated twice, each extraction lasting 4 hours. To the combined alkaline extract, ethanol was added until precipitation ceased. The precipitate was redissolved in 1-percent aqueous sodium hydroxide solution and re-precipitated with ethanol. The hemicellulose preparation thus obtained was very impure. It contained 13.1 percent of lignin and 8.1 percent of ash. No attempt was made to determine the composition of the hemicellulose.

METHODS AND RESULTS

DEPECTINIZATION

A portion of the cornstalks used in this investigation was ground in a Wiley mill, and the following constituents were determined [3] (in percent): Ash, 10.62; pentosans, 22.94; uronic acids (as anhydrides), 6.81. All results were calculated on the moisture-free basis.

The cornstalks used for the isolation of the hemicelluloses were cut into pieces about one-half inch long and extracted for 30 hours with a 1:2 ethanol-benzene solution in a large continuous copper extractor. This extractor was built on the same principle as the well-known Soxhlet extractor. The extracted cornstalks were ground in a Wiley mill fine enough to pass a 60-mesh sieve, and a portion of this material

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(765 gm.) was digested with 8 liters of hot (85° C.) water for 3 hours. The mixture was stirred manually from time to time during this heating operation. The product was filtered and the above-described extraction with hot water was repeated twice. In the last two extractions, however, only 600 cc. of hot water was used for each extraction. The three aqueous extracts were combined and evaporated to dryness on the steam bath. The dry extract weighed 50.6 gm., which was 6.6 percent of the weight of the dry ethanol-benzene-extracted cornstalks. The dry extract was analyzed for pectin by the Emmett and Carré (*5*) modification of the Carré and Haynes (*3*) method. It yielded a calcium pectate precipitate that weighed 0.5 percent of the weight of the ethanol-benzene-extracted moisture-free cornstalks.

The ground cornstalks, which had been extracted with hot water, were treated with 6 liters of 0.5-percent aqueous ammonium oxalate solution, and the mixture was digested at 85° C. for 4 hours, with manual stirring at frequent intervals. It was then filtered, and the residual material was digested twice with 0.5-percent aqueous ammonium oxalate solution, the procedure given above being followed. The insoluble material was filtered off, and it was then digested with two 6-liter portions of hot water, the digestion period being in each case approximately 1 hour. The 0.5-percent ammonium oxalate and hot water extracts were combined and concentrated under reduced pressure. To the concentrated solution four times its volume of 95-percent ethanol containing some hydrochloric acid was added, and the precipitate, after drying, was analyzed for pectin by the method already referred to. The yield [4] of pectin (as calcium pectate) was only 0.11 percent.

PARTIAL DELIGNIFICATION

To the pectin-free cornstalks a sufficient quantity of a 2-percent solution of sodium hydroxide in 60-percent ethanol was added to cover the cornstalks completely, and the mixture was digested at room temperature for 24 hours. During this digestion period, the reaction mixture was stirred mechanically. The plant material was filtered off, and the extraction with alcoholic sodium hydroxide solution was repeated twice. The combined alcoholic extract was neutralized with hydrochloric acid, and the ethanol was distilled under reduced pressure. The residual solution was made acid with hydrochloric acid, and the lignin was filtered. Neither the lignin nor the filtrate from it (treated as described in a previous publication (*11*)) afforded any furfural when distilled with 12-percent hydrochloric acid, thus indicating that in all probability this method of delignification did not bring about any loss of hemicellulose (*9*).

ISOLATION OF THE CRUDE HEMICELLULOSES

The material that had been extracted with alcoholic sodium-hydroxide solution was placed on the steam bath, and the alcohol was removed by evaporation. It was then mixed with sufficient 5-percent aqueous sodium hydroxide solution to make a thin suspension, and was allowed to digest at room temperature for 24 hours. It was then

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[4] All yields recorded in this paper were calculated on the basis of moisture-free and ethanol-benzene-extracted stalks.

filtered, and the above-described digestion with 5-percent aqueous sodium hydroxide solution was repeated three times. The combined alkaline extract amounted to 18.3 liters. To this was added sufficient 95-percent ethanol to make a 60 percent by volume ethanol solution. After being thoroughly mixed, the solution was allowed to stand at room temperature for 24 hours. The supernatant solution was drawn off, and the precipitate of hemicelluloses was mixed with sufficient 70-percent ethanol to make a thin suspension, from which the hemicelluloses were separated with the aid of the centrifuge. This treatment with 70-percent ethanol was repeated twice. The hemicellulose precipitate was then mixed with a fresh portion of 70-percent ethanol, and sufficient concentrated hydrochloric acid was added to make the mixture slightly acid. This was allowed to stand for several hours in order to insure complete neutralization, after which the acidified ethanol solution was removed with the aid of the centrifuge. The precipitate of crude hemicelluloses was then washed with three portions of 70-percent ethanol and with several portions of 95-percent ethanol. It was finally washed successively with two portions of absolute ethanol and one portion of anhydrous ether. It was dried in vacuo at 50° C. The yield amounted to 21 percent. Analysis of the product by the method of Goss and Phillips (6) showed that it contained 2.73 percent of lignin.

DELIGNIFICATION OF THE HEMICELLULOSES

The delignification of the hemicelluloses was accomplished by a modification of the procedure described in a previous publication (13). The crude hemicellulose preparation was placed in centrifuge bottles surrounded with ice. A current of chlorine was passed through the bottles for 2 hours. The bottles were then stoppered and allowed to remain in the refrigerator at a temperature of 8° C. for 4 to 5 hours. The material was washed twice with 95-percent ethanol and then extracted with a 3-percent ethanolamine solution in 95-percent ethanol. The product was washed successively with 95-percent ethanol, absolute ethanol, and anhydrous ether, and then dried in vacuo at 50°. The yield was 17 percent.

Analysis of the delignified product gave the following results (in percent): Pentosans, 83.51; uronic acids (as anhydrides), 7.73; ash, 1.95; methoxyl, 0.63; lignin, none.

HYDROLYSIS OF THE LIGNIN-FREE HEMICELLULOSES

To 45 gm. of lignin-free hemicelluloses, 2.5 liters of 2.5-percent sulfuric acid was added, and the mixture was boiled on an electric hot plate under a reflux condenser for 15 hours. After the reaction mixture had cooled, the dark precipitate was filtered on a weighed filter paper and dried at 105° C. The dried material weighed 0.70 gm., which was 1.5 percent of the weight of the hemicelluloses. To the filtrate approximately nine-tenths of the calculated quantity of barium hydroxide solution was added slowly, with stirring, while the temperature of the reaction mixture was kept at 40°. An excess of barium carbonate was then added, and the mixture was heated at 70° to 80° until neutral to litmus. Some Norit and Filter Cel were added to this, and after standing overnight, the mixture was filtered. The filtrate was concentrated to a volume of approximately 100 cc.

under reduced pressure at a temperature not exceeding 45°. The concentrated solution was poured slowly, with stirring, into 4 volumes of absolute ethanol, and the precipitated barium salt was separated with the aid of the centrifuge. The barium salt precipitate was dissolved in 25 cc. of water; the solution was decolorized with Norit and filtered; and the filtrate was poured into 4 volumes of absolute ethanol. The precipitate was again separated with the aid of the centrifuge, was washed successively with absolute ethanol and with anhydrous ether, and was dried in vacuo at 50°. The barium salt weighed 1.7 gm.

The supernatant alcoholic solution from the barium salt was concentrated under reduced pressure at 50° C. to remove the ethanol, and the thin sirup was transferred quantitatively to a 500-cc. volumetric flask and made up to the mark with distilled water. The total reducing sugars in this solution as determined by the method of Munsen and Walker (*1*) amounted to 33.5 gm. (calculated as glucose). Five cubic centimeters of the sugar solution, when distilled with 12-percent hydrochloric acid according to the procedure used for the determination of pentosans (*1*), afforded 0.3413 gm. of phloroglucide precipitate. This sugar solution contained a total of 8.48 gm. of arabinose, as determined by the Wise and Peterson (*14*) modification of the method of Neuberg and Wohlgemuth (*8*).

The remainder of the sugar solution was concentrated under reduced pressure to a thin sirup, which was allowed to remain in a desiccator over anhydrous calcium chloride until a considerable quantity of the sugar had crystallized. When filtered off and recrystallized from dilute ethanol, the sugar weighed 7.4 gm. It was identified as *d*-xylose by Bertrand's (*7*) method. The refractive indices of the double cadmium salt were found to be identical with those of the salt prepared by the same method from a known specimen of pure *d*-xylose.[5]

The sirup remaining after the separation of the crystals of *d*-xylose contained *l*-arabinose (identified as the diphenylhydrazone). No mannose, galactose, or fructose could be detected.

The barium salt obtained in the hydrolysis of the hemicellulose preparation described above gave Tollens' (*12*) naphthoresorcinol test for uronic acids. On oxidation with either nitric acid or bromine water, it did not form mucic acid, thus indicating the absence of galacturonic acid. The cinchonine salt as well as the brucine salt was prepared from the barium uronate according to the procedure described in a previous publication (*4*). The melting points of these two salts indicated that the uronic acid was *d*-glucuronic acid.

The analytical data (calculated on the moisture-free and ash-free basis) and the percentage composition of the hemicellulose preparation calculated from these data are as follows (the calculations were made as described in a previous publication (*13*)): Uronic acid (as anhydride), 7.88; total furfural, 49.81; furfural from uronic acid, 1.50; *l*-arabinose, 23.00; furfural from *l*-arabinose, 11.04; furfural from *d*-xylose (by difference), 37.27; *d*-xylose (calculated from furfural), 64.70; molar ratio of uronic acid to *l*-arabinose and *d*-xylose, 2:7:19.

The cellulosic material which remained after the exhaustive extraction with 5-percent aqueous sodium hydroxide solution and which still contained furfural-yielding constituents (10.4 percent, calculated as pentosans) was next treated with 5 liters of 10-percent aqueous

sodium hydroxide solution, and the mixture was stirred mechanically at room temperature for 7 hours. The mixture was then filtered, and the extraction operation described above was repeated twice. The residual cellulosic material obtained from the third extraction was washed free of alkali and dried. It contained 5.87 percent of pentosans and 1.40 percent of uronic acid (as anhydride).

To the alkaline filtrate sufficient 95-percent ethanol was added to make a 60-percent alcohol solution. The hemicellulose precipitate was separated with the aid of the centrifuge and washed successively with neutral 70-percent ethanol, with 70-percent ethanol acidified with acetic acid, with graded strengths [6] of ethanol, and finally with ether. After it was dried in the vacuum oven at 50° C., it weighed 13.85 gm. An analysis of this material gave the following results (in percent): Pentosans, 64.45; uronic acid (as anhydride), 5.09; methoxyl, 1.12; ash, 0.54.

A portion (172 gm.) of the cellulosic residue remaining from the extraction with the 10-percent aqueous sodium hydroxide solution was treated with 2 liters of 17-percent aqueous sodium hydroxide solution and allowed to remain at room temperature for 2 days. From time to time the reaction mixture was stirred manually. It was filtered, and the residual cellulosic material was again digested with 17-percent aqueous sodium hydroxide solution for 2 days. The residual material from the second extraction was thoroughly washed with cold and hot water and then dried at 105° C. It weighed 38.5 gm. and contained 2 percent of pentosans and 1.20 percent of uronic acids (as anhydride).

The alkaline extracts and washings from the two extractions with 17-percent aqueous sodium hydroxide solution were combined, and the hemicelluloses were isolated, the procedure already described being used. The yield amounted to 10.7 gm. The uronic acids (as anhydrides) and the furfural-yielding constituents, calculated as pentosans, in this hemicellulose fraction amounted to 3.62 and 49.34 percent, respectively.

HYDROLYSIS OF THE HEMICELLULOSE FRACTIONS FROM THE 10-AND 17-PERCENT AQUEOUS SODIUM HYDROXIDE EXTRACTIONS

The hydrolysis of the hemicellulose fractions from the 10- and 17-percent aqueous sodium hydroxide extractions was carried out in the manner already described. In both cases, the only sugars identified were *d*-xylose and *l*-arabinose. Because of the small quantity of barium uronate obtained, it was not possible to identify the uronic acid in these two hemicellulose fractions.

SUMMARY

A hemicellulose fraction was isolated from cornstalks, which had previously been freed of fatty and waxy materials, sugars, and pectic substances, by extracting them exhaustively at room temperature with a 5-percent aqueous sodium hydroxide solution and precipitating with ethanol. The product was delignified by treatment with chlorine and extraction with an ethanolamine solution in ethanol. On hydrol-

[6] The term "graded strengths" as used in this paper means 70-percent, 85-percent, 95-percent, and absolute ethanol

ysis with dilute sulfuric acid, the lignin-free hemicellulose fraction afforded *d*-glucuronic acid, *l*-arabinose, and *d*-xylose in the approximate molar ratio of 2:7:19.

The cellulosic material remaining after the removal of the first hemicellulose fraction was successively extracted in the cold with 10- and 17-percent aqueous sodium hydroxide solutions, and two additional hemicellulose fractions were obtained. These differed quantitatively from the first hemicellulose fraction, although on hydrolysis they both yielded *d*-xylose and *l*-arabinose.

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FACTORS AFFECTING THE LONGEVITY OF COTTONSEED [1]

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INTRODUCTION

Exposed cottonseed gradually attains moisture equilibrium with the surrounding atmosphere. This point of equilibrium varies with the storage environment and is dependent largely upon local climatic conditions. Approximately 11 percent of moisture (wet-weight basis) is common in stored seeds in the coastal areas of the Cotton Belt, and about 8 percent or less in the semiarid regions of Texas and other western cotton-growing States. Minor fluctuations may occur, especially with extended periods of low or high relative humidity, but once a condition of equilibrium is reached the seed moisture is comparatively constant in a given locality.

Experiments have shown that rapid loss of viability occurs in stored cottonseed containing excessive moisture and that storage temperature is an important factor affecting the moisture tolerance of seeds. Little information is available, however, concerning the tolerance of cottonseed to extremes of moisture or temperature or to the effects produced by the interaction of moisture and temperature. These factors are important not only to investigators working with cotton but also to farmers, seedsmen, and others who have occasion to store cottonseed for planting or milling purposes.

REVIEW OF LITERATURE

In a comprehensive study of the viability and germination of seeds, Duvel (1) [2] concluded that the longevity of any species of seed thoroughly matured and cured and properly harvested is dependent on storage environment and that moisture is the chief factor in determining longevity; furthermore, that the deleterious action of moisture is greatly augmented if temperature is increased. Eastham (2) in Canada, Welton (16) in Ohio, and Robertson and Lute (9, 10) and Robertson, Lute, and Gardner (11) in Colorado have stored farm-crop seeds for periods of 10 to 15 years and found that the extent of deterioration varied with different crops and with the relative humidity of the storage environment. Sonavne (14) reported a general belief in India that seeds more than 1 year old do not germinate satisfactorily, but stated that in his experiments seeds sealed in bottles gave excellent germination after 5 years' storage. In experiments with wheat, Kelly (4) found that under typical storage conditions low moisture content was the most important requirement for safe storage, and that in locations having lower temperatures a higher moisture content was permissible. At James Island, S. C., the author (12) found that in ordinary storage, under humid con-

ditions, cottonseed deteriorated rapidly after 2 years and that a definite relation existed between moisture content of seeds during storage and rapidity of deterioration. Somewhat similar results have been reported by Flores (*3*) in the Philippines, but there cottonseed deteriorated more rapidly, exhibiting less tolerance for moisture than seeds stored under humid conditions in the coastal section of South Carolina. The writer suggests that these differences probably are temperature effects. Flores showed also that seeds stored as unginned cotton retained their vitality longer than ginned or acid-delinted seeds.

MATERIALS AND METHODS

In every case the seeds used in these studies were from the crop immediately preceding the experiment and were classed as fresh seeds. Both upland and sea-island seeds were employed in certain of the experiments. Sea-island seeds, which were used for storage in burlap bags and metal containers, were approximately 8 months old when placed in storage under the controlled conditions of the experiment. These seeds had been in ordinary dry storage near Charleston, S. C., during the 8 months prior to the beginning of the experiments.

Determination of moisture percentages was accomplished by the weighing of samples before and after drying in an electric oven for approximately 24 hours, at a constant temperature of 100° C. Samples were placed in a desiccator to cool before final weighing. Moisture content lost in drying is expressed as percentage of the original weight—in other words, on a wet-weight basis. Conversion from a wet- to a dry-weight basis may be made by using the following formula:

$$\frac{\text{Moisture percent (wet basis)}}{100 - \text{moisture percent (wet basis)}} \times 100 - \text{moisture percent (dry basis)}$$

Germination percentages, except in field tests, were determined in the laboratory by the standard method for the germination of cottonseed as recommended by the Association of Seed Analysts of North America and described by Toole and Drummond (*15*).

In the experiments in which moisture content of seeds was adjusted, initial moisture was first determined. To obtain a moisture content higher than the initial, a calculated amount of water was added to a weighed sublot of seed, which was then mixed thoroughly and placed in a moistureproof container. Seeds were mixed several times before making final moisture determinations prior to storage. Lower levels of moisture were obtained by drying the seeds at a low temperature until the desired levels were reached. Moisture determinations that have been made on individual samples since storage have shown very little variation among subsamples of the same lot, even after several years' storage.

EFFECT OF STORAGE METHOD ON LONGEVITY

A series of tests was begun near Charleston, in July 1930, in which air-dried and sun-dried sea-island cottonseed from the crop of 1929 was stored in burlap bags and metal containers. Ordinary storage conditions near Charleston are less favorable for seed longevity than those prevailing in most sections of the Cotton Belt. The average annual

temperature at Charleston is 65.6° F., and the average annual relative humidity is 79 percent at 8 a. m. and 77 percent at 8 p. m. A report of these tests made in 1934 (*12*) showed that seed moisture is a critical factor in longevity of stored seeds. It was also shown that sun-dried seeds stored in burlap bags rapidly regained moisture to approximately 11 percent. Seeds stored in bags deteriorated rapidly after 2 years and were all dead approximately 3 years after storage. In contrast, seeds sun-dried to approximately 8 percent moisture and stored in metal containers were still in excellent condition when the report was made in 1934.

This series of tests has been continued with the same seeds in storage near Charleston, S. C., and determinations of moisture and germination have been made at intervals. Part of the data of the first report and subsequent determinations are given in table 1. Sun-dried seeds stored in cans unsealed or with perforated lids gradually reabsorbed moisture and showed some deterioration after 4 years' storage and were worthless for planting purposes when tested in September 1937. Seeds sun-dried and sealed in cans reabsorbed moisture more slowly and showed only slight deterioration after 7 years' storage. These cans, though sealed with tight lids, were opened at intervals for sampling; thus an interchange of air was possible and a slow increase in moisture percentage occurred. Since 1937, deterioration has continued; in April 1940, only a few weak seedlings were obtained in lot 6.

In July 1934, portions of seed lots 4, 5, and 6 were redried to approximately 7.0 percent moisture and placed in separate metal cans. These redried lots are indicated in table 1 as 4A, 5A, and 6A, respectively. Comparison of redried lots with those not redried indicates that only minor improvement was obtained by redrying and that the longevity of the seeds was not increased materially. In field tests of these seeds in 1940, lot 6 produced 1 plant from 400 seeds, while lot 6A produced 30 plants from 400 seeds. The seeds were 10½ years old when planted. These records indicate that for long-time storage at ordinary temperature the moisture percentage must be held below 9.0 percent and that cotton seeds deteriorate rapidly when the moisture is much above that point.

The foregoing experiment illustrates a practical means of safely storing cottonseed for several seasons despite unfavorable climatic conditions. If air-dried seeds are spread in a thin layer on a dry solid surface and exposed to direct sunshine for approximately 7 hours, the moisture content of the seeds after drying will not be likely to exceed 8 percent. Sun-drying is most effective during warm weather and on days of low relative humidity. A tight wooden platform or flat metal roof makes an excellent drying arbor. Metal drums with tight-fitting lids, such as are used for shipment of insecticide dusts, usually are obtainable and make excellent storage containers. Treated seed or seed stored in drums that have formerly contained posionous substances should be colored if possible and the containers should be plainly marked, "Poison, do not sell for crushing or mix with other seed." Sun-dried seeds stored in this way may be kept for 6 or 8 years and still retain viability. Cans may remain tightly closed or even sealed, since aeration is not necessary for proper storage of dry cottonseed. Apparently dry seeds treated with Ceresan may be stored under similar conditions as untreated seed, if proper precautions

are taken. Miles (*8*) reports that seeds treated before storage are superior in performance to untreated seeds similarly stored for the same length of time.

TABLE 1.—*Moisture content and germination percentage of sea-island cottonseed air-dried and sun-dried and stored in cans and bags near Charleston, S. C., July 1930 to November 1940*

	Moisture content and germination of cottonseed after—																			
	Air-drying and storage in—						Sun-drying and storage in—													
	Cans with lid—				Bags		Cans with lid—												Bags	
	Perforated		Closed, unsealed				Perforated				Closed but unsealed				Sealed after each sampling					
									Redried, 1934				Redried, 1934				Redried, 1934			
Date of test	Lot 1		Lot 2		Lot 3		Lot 4		Lot 4A		Lot 5		Lot 5A		Lot 6		Lot 6A		Lot 7	
	Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination	Moisture	Germination
	Pct.	Pct.	Pct.	Pct.	Pct.	Pct	Pct	Pct	Pct.	Pct.	Pct	Pct	Pct.	Pct	Pct	Pct	Pct.	Pct.	Pct	Pct
1930 July	10 8	90	10 8	90	10.8	90	7.8	85	..	..	7 8	85	.		7 8	85	..		7.8	85
1932 March	11.2	79	11.6	84	11 3	88	9 0	84		.	8-4	87	.		8.4	91	..		11.0	85
October	11.5	48	11.3	39	11.7	45	9.0	84		..	9.3	86			8.4	87	.		11.2	64
1933 April	11 2	34	11 5	19	11.3	17	9.2	81	.		9 6	89		..	8.2	82		.	11.2	38
October	11 2	0	11 3	0	11 3	0	9 3	80	..	..	9 2	77	..	.	8 2	81			10.9	0
1934 May	11.4	0	11.6	0	11.1	0	9.3	83	..		9.2	90	..	..	8.2	86		..	10 7	0
July							9.3	84	7 0	75	9 2	78	7.1	87	8.2	89	6.6	91		.
August							9 4	62	7.8	72	9.1	84	7.7	80	8.2	88	7 1	84		
October							9 3	63	8.0	81	9 1	79	7 9	75	8.0	83	7 0	87		
1937 September		.					10.9	0	10 7	0	9.7	14	9.2	36	8.9	72	8.3	72		
1938 January							10 5	0	10 9	0	9 9	6	10 0	44	9 3	69	8.5	68		
September											9.5	0	9 2	0	8 8	43	8.1	76		
1939 January													.	..	8 9	42	8.3	61	..	.
July											..	.			9 0	24	8 2	50		
1940 April	.	..				..								..	9 7	8	8 8	34	..	
November										..	..	..		..	9.5	0	8.8	8		

EFFECT OF MOISTURE CONTENT ON LONGEVITY

The foregoing experiment has shown the rate of deterioration of sea-island cottonseed stored at atmospheric temperature in relatively large quantities and in containers in which replacement of air was possible at frequent intervals. In other tests, small quantities of seeds have been brought to predetermined levels of moisture and placed in glass jars, sealed, and stored. A sufficient number of samples were prepared in individual jars, so that jars once opened for

sampling were not used further in the experiment. A test of this type was begun near Charleston, S. C., in January 1932, with upland cottonseed containing 9.5 percent moisture. These seeds were continued in storage near Charleston until the conclusion of the test in January 1939.

Results of this test (table 2) show that deterioration was slight for the first 2 years of storage, but thereafter the seeds steadily decreased in germination percentage until all were dead when tested in September 1938, 6½ years after storage. The few seeds that germinated in January 1938 were weak and probably would not have survived except under optimum conditions.

TABLE 2.— *Moisture content and germination percentage of upland cottonseed stored Jan. 10, 1932, in sealed glass jars at approximately 9.5 percent moisture, Charleston, S. C.*

Date of test	Moisture	Germination	Date of test	Moisture	Germination
	Percent	*Percent*		*Percent*	*Percent*
January 1932	9 5	83	March 1936	9 6	23
July 1932	9 7	72	July 1936		25
January 1933	9 7	73	March 1937		36
July 1933	9 7	66	August 1937	9 5	[1] 19
January 1934	9 8	69	January 1938	9 7	[1] 12
January 1935		50	September 1938	9 5	0
July 1935	9 0	48	January 1939	9.8	0

[1] Weak.

Other tests with upland cottonseed stored in sealed glass jars were begun in December 1932. Seeds of the same original lot were subdivided and portions brought to moisture levels of approximately 6, 8, and 11 percent. A number of samples of each moisture content were prepared to provide material for testing at intervals over a period of years. The results of these tests (table 3) show that seeds containing 11 percent of moisture had seriously deteriorated in 1 year and were worthless for planting purposes after 2 years' storage. These seeds were all dead 3½ years after storage. Seeds containing 8 percent and 6 percent moisture, after 2 years' storage showed some loss of viability, but the decrease was slow, and the samples still contained many viable seeds after 7½ years' storage. In field germination tests in May 1940, 50 percent of the seeds stored with 6 percent moisture germinated, and 52 percent of the seeds with 8 percent moisture germinated. In these field plantings, seedlings were comparatively free from disease lesions on the roots; none of the seedlings died between emergence and thinning time.

A test similar to that described above was begun in February 1933 with sea-island cottonseed of the 1932 crop. The results of this test (table 3) are similar to those obtained with upland cottonseed. In the seeds containing 11 percent of moisture no deterioration was apparent at the end of the first year of storage, but within 1½ years definite deterioration had occurred and within 2 years all seeds were dead. Seeds stored with 6 percent and 8 percent of moisture, on the other hand, showed only slight impairment in 7½ years of storage. In field germination tests in May 1940, 69 percent of sea-island seeds stored with 6 percent moisture and 69 percent of seeds stored with 8 percent moisture germinated. The seedlings produced by these seeds were exceptionally healthy and were free from root lesions.

TABLE 3.—*Moisture content and germination percentages of upland cottonseed (Tidewater) stored Dec. 14, 1932, and of sea-island cottonseed (Seabrook) stored Feb. 10, 1933, in sealed glass jars at approximately 6, 8, and 11 percent moisture, Charleston, S. C.*

UPLAND

Date of test	Cottonseed stored at—					
	11 percent moisture		8 percent moisture		6 percent moisture	
	Moisture	Germination	Moisture	Germination	Moisture	Germination
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
December 1932	11.0	79	8.2	74	6 2	80
January 1933	10 7	59	7 9	62	6 1	71
December 1933	11 2	47	8 5	79	6 2	72
June 1934	11 3	45	8 2	83	6 3	72
January 1935		12		62		71
June 1935	10 7	13	7.9	58	6 0	69
December 1935	11 3	4	8 3	63	6 4	65
June 1936		0		66		62
August 1937	11 3	0	8.0	50	6 1	63
January 1938			8.4	57	6 6	58
September 1938			8 3	38	6 1	57
January 1939			8 2	42	6 5	46
July 1939			8.0	47	6 3	62
April 1940			8 4	53	6 6	49
November 1940			8.4	46	6 5	44

SEA-ISLAND

Date of test	11 percent moisture		8 percent moisture		6 percent moisture	
	Moisture	Germination	Moisture	Germination	Moisture	Germination
February 1933	11 2	90	7.5	94	5 6	89
August 1933	10 9	91	7 1	94	5 2	92
February 1934	12 0	93	8 3	91	6 5	97
August 1934	11.1	61	7 3	97	5 5	91
February 1935		0		89		91
December 1935	11.4	0	7.5	85	5 9	85
March 1936	11.4	0	7.6	93	5 8	88
August 1936				86		88
March 1937				88		85
August 1937	11 2	0	7 3	90	5 9	81
February 1938			7.5	89	6 1	88
September 1938			7.4	86	6 4	81
January 1939			7 5	94	5.7	89
July 1939			7 3	88	5 9	77
April 1940			7.6	84	6 1	84
November 1940			7 8	90	5 8	81

RELATION OF TEMPERATURE AND MOISTURE TO LONGEVITY

In addition to ascertaining the injurious effect of high moisture content upon the longevity of cottonseed it seemed desirable to determine the effect of storage temperature on viability, particularly as related to moisture tolerance at different temperature levels.

For the study of these related factors in seed deterioration, bulk seeds of two varieties of cotton were obtained in 1937 from gins near Clemson, S. C., for storage tests. One of the lots was of the Deltapine A variety and was obtained from the gin within 3 days after picking. This lot had an initial moisture content of 13.5 percent. The second lot of seed, Carolina Dell, was obtained from the gin within 10 days after picking and had an initial moisture content of 11.5 percent. Bulk seeds of each variety were subdivided into five lots and the lots moistened or dried to obtain certain desired moisture-percentage levels. These levels approximated 7, 9, 11, 13, and 14 percent (table 4). When these moisture percentages had been obtained, samples of each lot were sealed in glass jars and placed in storage at various temperatures.[3]

[3] This experiment was planned and is being conducted in cooperation with Dr. C. H. Arndt, of the South Carolina Agricultural Experiment Station. The seeds were prepared and sealed in glass jars by Dr. Arndt at Clemson, S. C., from which point they were shipped to Knoxville, Tenn., for storage. Samples are being returned to Dr. Arndt at intervals for a study of the micro-organisms associated with the seeds.

TABLE 4.—*Initial germination percentage and moisture content (as determined from samples taken in 1937 and 1939) of upland cottonseed used in moisture-temperature seed-storage tests*

Variety	Initial germination, December 1937	Moisture content						
		December 1937, sample No.		December 1939, sample No —				Average of all samples
		1	2	1	2	3	4	
	Percent	Percent	Percent	Percent	Percent	Percent	Percent	Percent
	92	6 7	7 7		7 6	7 3	6.1	7.1
	90	9 2	8 9	8 6	8.7	8 7	8 6	8.8
Carolina Dell	91	10.7	10 9	10 6	10 6	10 6	10.4	10.6
	77	12 4	12 9	12 7	12 4	12 5	12 7	12.6
	90	14 2	14 3	14 8	13 9	14.7	16 4	14.7
	88	7 1	8 2	7 7	7 7	7.7	7 2	7.6
	91	9 2	10 0	8 9	9 6	9.6	9 5	9.5
Deltapine A	83	10 7	11 2	10 8	10 8	10 8	10 5	10 8
	83	12 2	12 4	12 4	12 3	12 3	12 6	12.4
	83	13 5	14.0	14 9	13.6	14 3	14 7	14 2

The conditions of storage provided three relatively constant temperatures and an uncontrolled check: (1) Low-temperature oven (90° F.); (2) air-conditioned room (70°); (3) commercial cold storage (33°); (4) check, unheated metal seed-storage house (uncontrolled air temperature).

A number of samples of each lot were stored under each of the four conditions to provide material for testing at intervals over a period of years. Initial germination and moisture tests of these various lots of seed were made in December 1937 (table 4). At that
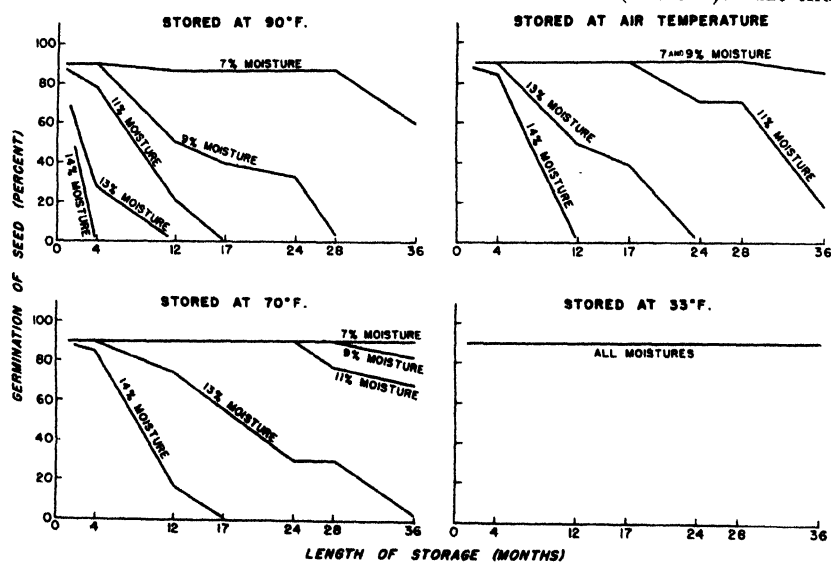
FIGURE 1.—Graphic summary of table 5, with varieties combined. Minor variations that did not seem to represent definite trends have been ignored.

time all lots were relatively high in germination, the percentage averaging 88 for Carolina Dell and 86 for Deltapine A. The seeds were placed in storage at Knoxville in December 1937 and have been tested for germination at intervals during the 3-year storage period. The results of these laboratory germination tests are given in table 5 and figure 1.

TABLE 5.— *Effect of moisture content and storage temperatures upon the viability of cottonseed from 2 upland varieties, stored in sealed containers on Dec. 8, 1937, for the duration indicated by each recording of germination for the moisture-temperature combinations shown*

Storage conditions			Germination of seed after storage for indicated number of months					
Temperature (° F)	Approximate moisture content	Variety	4	12	17	24	28	36
	Percent		*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
90	7	Carolina Dell	89	88	88	87	90	53
	9		95	87	46	65	5	0
	11		88	29	0	0	...	...
	13		51	0	0	..	...	...
	14		0	0			..	
	7	Deltapine A	94	84	81	84	84	65
	9		89	13	32	0	0	0
	11		68	13	0	0	...	..
	13		4	0	0		...	..
	14		0	0				
Air (uncontrolled)	7	Carolina Dell	95	90	97	88	84	85
	9		95	93	91	85	93	91
	11		82	83	82	54	67	5
	13		91	35	2	0	0	
	14		88	0	0			
	7	Deltapine A	86	83	86	91	91	81
	9		89	88	91	91	85	76
	11		85	87	88	84	74	30
	13		90	62	76	0	0	
	14		78	0	0			
70	7	Carolina Dell	96	95	96	92	88	88
	9		91	88	90	93	90	82
	11		97	89	93	83	77	57
	13		89	65	70	17	4	0
	14		86	5	0	0		
	7	Deltapine A	88	90	87	90	88	91
	9		89	86	94	88	89	82
	11		91	83	94	94	77	79
	13		90	78	78	29	55	6
	14		83	29	1	0		
33	7	Carolina Dell	88	87	90	96	88	92
	9		96	91	90	89	94	93
	11		95	85	93	91	89	90
	13		87	87	89	91	95	89
	14		86	90	92	89	89	88
	7	Deltapine A	90	86	84	77	88	82
	9		89	92	88	85	86	92
	11		87	93	83	84	88	92
	13		84	92	81	83	94	82
	14		86	86	88	90	82	81

STORAGE AT 90° F.

For storage at a constant temperature of 90° F., several samples of both varieties at each moisture content indicated (table 5) were placed in an insulated low-temperature oven having thermostatic control. The first tests after storage were made in April 1938. The Carolina Dell lots having 7, 9, and 11 percent moisture showed no deterioration at that time, but seeds having 13 percent moisture had dropped to 51 percent germination and seeds having 14 percent moisture were all dead. The Deltapine A seeds having 7 and 9 percent moisture had not deteriorated after 4 months' storage, but seeds having 11 and 13 percent moisture had dropped to 68 and 4 percent germination, respectively. Seeds having 14 percent moisture were dead.

In November 1938, 12 months after storage, Carolina Dell seeds having 7 and 9 percent moisture were still high in germination, but seeds having 11 percent moisture had dropped to 29 percent germination, and seeds having 13 and 14 percent moisture were dead.

Deltapine A seeds having 7 percent moisture were still high in germination, those having 9 and 11 percent were badly deteriorated, and those with 13 and 14 percent were dead.

With both varieties, after 17 months' storage, seeds having 7 percent moisture were still good, while seeds with 9 percent moisture were considerably deteriorated, and those with higher moisture content were all dead. In December 1939, 2 years after storage, only those seeds with 7 percent moisture remained in good condition; in the lot of Carolina Dell at 9 percent moisture, 65 percent of the seeds produced radicles but many seedlings were not vigorous. After 36 months' storage, only the seeds with 7 percent moisture were germinable, and these did not produce altogether normal seedlings.

STORAGE AT AIR TEMPERATURE

The seeds stored at air temperature were placed in an unheated metal building and were subject to the normal fluctuations of temperature within the building. Temperature records were not maintained in the building, but summer temperatures probably exceeded 100° F. on occasions, and winter temperatures frequently have been below zero. The annual average temperature at Knoxville, Tenn., is 58.4°. This storage temperature would approximate very closely that obtainable in ordinary farm storage over the major part of the Cotton Belt of the piedmont section.

None of the seeds stored at air temperature showed appreciable deterioration in 4 months, but those lots containing more than 11 percent moisture deteriorated rapidly thereafter and were all dead in 24 months. Seeds containing about 11 percent moisture showed some deterioration in 24 months and were worthless for planting purposes after 36 months' storage. Seeds containing 7 and 9 percent moisture were still in good condition after 36 months' storage.

STORAGE AT 70° F.

None of the seeds stored at 70° F. showed appreciable deterioration in 4 months. In 12 months, seeds of Carolina Dell and Deltapine A having 13 percent moisture dropped to 65 and 78 percent germination, respectively, and seeds with 14 percent moisture dropped to 5 and 29 percent germination. After 17 months' storage, no further decrease in germination was noted in the seeds having 13 percent moisture, but in those having 14 percent moisture germination was 0 and 1 percent respectively. Seeds having 7, 9, and 11 percent moisture were still in good condition 24 months after storage, but those with 13 percent moisture had dropped to 17 and 29 percent germination with poorly developed seedlings.

After 36 months, seeds containing 7 and 9 percent moisture were still in excellent condition, but some deterioration was evident in samples containing 11 percent moisture, and seeds containing 13 and 14 percent moisture were worthless for planting.

STORAGE AT 33° F.

With seeds stored in commercial cold storage at approximately 33° F. all lots of seed, irrespective of moisture content, showed no appreciable impairment after storage for 36 months.

It is felt that the remarkable results secured at 33° F., plus the

striking differences obtained for moisture levels within and between the three other storage temperatures, furnish conclusive proof of the interaction of moisture and temperature in relation to cottonseed deterioration.

SEEDLING MORTALITY IN FIELD TESTS OF STORED SEEDS

All of the preceding germination percentages have been determined in the laboratory by standard methods for cottonseed germination. Inasmuch as weakened but still germinable seeds often are difficult to detect by laboratory methods, field germination tests were made, in May 1940, as a further check on the viability and vigor of the stored seeds. At that time the seeds had been in storage 2½ years. The results of these tests are given in table 6. The germination percentages obtained in the field were considerably below those obtained in the laboratory, but this is in accord with the usual results with cottonseed. In general, the field behavior coincided with expectations based on the laboratory germination percentages, except in the case of the seed lots stored at 33° F. These lots were somewhat lower in field germination than other lots of approximately the same laboratory germination percentage stored at higher temperatures. Post-germination mortality also was comparatively high among seedlings from lots stored at 33°. The reason for this cannot be stated definitely, as the causal organisms were not determined. It is known, however, that prior to storage the various seed lots showed some contamination with the anthracnose organism and that after 19 months' storage anthracnose spores were still alive and capable of causing infection [4] in the lots stored at 33°. It is logical to assume that the storage temperature of 33° was more favorable for the survival of the anthracnose spores as well as for the cottonseed than were the higher temperatures, and that these results corroborate the

TABLE 6.—*Field germination and mortality of emerged seedlings from upland cottonseed stored for 2½ years at 90° F., air temperature, 70°, and 33°, Knoxville, Tenn.*

Storage conditions		Carolina Dell		Deltapine A	
Temperature (°F)	Approximate moisture content	Total emergence [1]	Mortality of emerged seedlings	Total emergence [1]	Mortality of emerged seedlings
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
90	7	68 5	0 0	67.0	0 7
	9	0		.0	
	7	75.0	.7	75 5	.0
	9	85 0	1.8	68 5	2. 9
Air (uncontrolled)	11	39 0	1.3	32.0	.0
	13	.0		.0	
	14	0		.0	
	7	78 0	1 9	69 0	.7
70	9	73.5	.0	75 0	.0
	11	62 5	1.6	66.5	.0
	13	6 5	.0	18 5	.0
	14	.0		.0	
	7	60 0	10.8	59 0	25 4
33	9	67.0	14 9	59.0	22 0
	11	70 5	7 1	59.5	9.2
	13	60.5	5 0	64.5	17.8
	14	71 5	2 8	54.5	3.7

[1] Total emergence is on the basis of 200 seeds planted for each lot.

findings of Arndt and Boozer [4] in their studies of anthracnose survival in stored seeds.[5]

FREE FATTY ACIDS IN THE OIL IN STORED SEEDS

Cottonseed received at the oil mills frequently contains high percentages of free fatty acids which reduce its milling value. Seeds from cotton that has been exposed to wet weather in the field are likely to be lower in germinability (*13*) and to contain higher percentages of free fatty acids (*7*) than those from cotton harvested before unfavorable exposure.

In short-time laboratory tests, Malowan (*5, 6*) found that chemical changes in the seeds took place during heating; but he concluded that as long as the seeds did not heat practically no change took place. Meloy (*7*), in discussing delayed opening and field damage to cottonseed, expressed the belief that continued exposure to atmospheric humidity and high temperatures raises the free fatty acid content of the seed. However, when attempts were made to group Mississippi-grown seed on the basis of free fatty acid and seed moisture content, the importance of moisture was not substantiated. The writer feels that this finding should not be a cause for concern, since moisture content at the time of seed sampling would not necessarily indicate previous field history of moisture content for the seed.

Some specific information on the development of free fatty acids in cottonseed is afforded by the analyses [6] made on samples from the moisture-temperature storage tests, after 2 years' storage (table 7). These data show a rapid rise in free fatty acids with increased moisture and also with increased storage temperature. It is clearly apparent that moisture and temperature are important and interdependent factors in the development of free fatty acids in stored seeds.

TABLE 7. *Free fatty acids in oil of upland cottonseed stored December 1937, from moisture-temperature storage tests made after 2 years' storage*

Variety	Approximate moisture content	Free fatty acids in oil from seeds stored for 2 years at indicated temperature (°F.)				
		90	An	70	33	Average
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Carolina Dell	7 1	0 9	0 5	0 7	0 4	0 63
	8 8	1 1 7	9	8	4	95
	10 6	2 2 7	1 1 3	1 0	7	1 43
	12 6	2 10 0	2 2 6	1 1 8	6	3 75
	14 7	2 21 9	2 7 4	1 3 0	1 0	8 33
Average		7 41	2 54	1 46	62	3 02
Deltapine A	7 6	1 5	1 1	8	6	1 00
	9 5	2 2 6	1 0	9	7	1 30
	10 8	2 3 0	1 3	1 3	6	1 55
	12 4	2 9 5	2 2 8	1 1 4	6	3 58
	14 2	2 26 0	2 5 8	2 2 6	7	8 78
Average		8 52	2 40	1 10	64	3 24

[1] Deteriorated, germination below 66 percent.
[2] All seeds dead

[5] Subsequent field tests have shown that viable spores of anthracnose and *Fusarium moniliforme* were present on seedlings from lots stored at 33° F. after 3½ years' storage, but these organisms were not found on seedlings from lots stored at higher temperatures. The tests also indicate somewhat greater survival of the fungi at the lower moisture contents at 33°

If the amount of free fatty acids in the oil from these seed lots is considered in relation to the germination obtained from other portions of the same samples (see table 5, 24 months' storage), it is found that in all cases lots containing more than 1.8 percent free fatty acids in the oil failed to germinate; samples approaching that level germinated poorly and gave weak seedlings.

SUMMARY

The longevity of cottonseed is definitely dependent upon the moisture content of the seeds and the temperature conditions under which the seeds are stored. The studies here reported deal with the effects of moisture alone under "normal" storage temperatures and with the combined effects of controlled moisture-temperature conditions.

In ordinary storage, seeds quickly reach a moisture content in equilibrium with that of the storage environment. In storage experiments with upland and sea-island cottonseed under the humid and fairly high temperature conditions prevailing near Charleston, S. C., seeds in bags deteriorated rapidly after 2 years, but seeds with a moisture content reduced below 8 percent and stored in tin containers to prevent the rapid reabsorption of moisture retained their viability with only slight impairment for 7 years, and a few seeds were still germinable after 10 years' storage.

Lots of upland and sea-island cottonseed sealed in glass jars and containing 11 percent moisture were worthless for planting purposes after 2 years' storage, but other lots, especially of the sea-island seed, containing 6 and 8 percent moisture, showed a high percentage of viable seeds after 7½ years' storage. Thus, cottonseed containing less than 8 percent moisture apparently does not require aeration and can be kept viable for many years in airtight containers even at the temperatures that prevail along the Coastal Plain.

Cottonseed of two upland varieties was adjusted to several levels of moisture ranging from 7 to 14 percent and stored at constant temperatures of 90°, 70°, and 33° F. Corresponding checks were subjected to normal fluctuating temperatures at Knoxville, Tenn. The seeds stored at 90° deteriorated rapidly, those containing 14 percent moisture were all dead in 4 months, and after 36 months' storage only those seeds with 7 percent moisture were germinable, and their vitality was impaired. In contrast, seeds stored at 33°, even with 14 percent moisture, retained their viability for 36 months without appreciable impairment. Seeds stored at air temperature and at 70° were somewhat intermediate with respect to moisture tolerance. The higher moisture lots deteriorated less rapidly at 70° than at air temperature.

If the moisture content is low cotton seeds can withstand high temperatures without rapid deterioration, and if the temperature is kept low they are tolerant of high moisture, but both temperature and moisture cannot be high if rapid deterioration is to be prevented.

In field germination tests, the percentage of seedling mortality was greater from seeds stored at 33° F. than from seeds stored at higher temperatures. Apparently, the low storage temperature was also favorable for the survival of anthracnose spores on the seeds.

Analyses of stored seeds showed that with increased seed moisture

or increased storage temperature there was a corresponding increase in the percentage of free fatty acids in the oil.

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NATURAL MODE OF ENTRANCE OF FUNGI INTO CORN EARS AND SOME SYMPTOMS THAT INDICATE INFECTION [1]

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INTRODUCTION

Very little has been published regarding the time and manner in which fungi enter and permeate developing ears of corn (*Zea mays* L.). Since no very satisfactory way of controlling the corn ear rots is known, it seemed desirable to learn more about the actual course or path of infection. Such a study was started in the season of 1933 and continued during the seasons of 1935 and 1937. The work was suspended during 1934 because of severe chinch bug damage to the corn on the station farm, and little was accomplished in 1936 because of damage from drought.

TECHNIQUE

METHOD 1

The principal procedure was the plating of natural unsterilized pieces of tissue from different parts of the ear. The station strain of Reid Yellow Dent open-pollinated corn was used. Work was limited to ears that were 7 or more inches in length, were enclosed tightly by the husks, were not penetrated farther than the tip end by corn earworms (*Heliothis armigera* (Hbn.)), and did not already show visible signs of moldiness except sometimes a slight amount at the tip end. Otherwise, no selection of ears was made. Ears that were slightly exposed to the weather at the tip end only, or showed signs of corn earworm activity at the tip of the ear, with or without accompanying mold growth, were classified and handled separately from the ears that were well covered with husks and appeared entirely sound.

Ears were broken off with shank and husks intact at different stages, ranging from late-milk, average grain moisture 55.6 percent, to mature average moisture 24.9 percent (table 1). The outer husks were removed out of doors, leaving two or three layers of the clean inner husks to cover the kernels. The tips of the husks were then snipped off even with the tips of the ears and the ears were placed tip down in a large clean pail containing a 1:200 formalin solution 1 inch deep at the bottom. This process killed any mold at the tips of the ears and thus helped to prevent contaminations. The pail with corn ears was then covered with clean wet toweling and brought into the laboratory.

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TABLE 1.—*Fusarium moniliforme infection on or in kernels of Reid Yellow Dent corn at different times during kernel development, studied by method 1, 3-year average, Urbana, Ill., 1933, 1935, and 1937*

Date	Average moisture content of—		Condition of tip of ear	Ears in test	Ears with infected kernels—	
	Grain	Cob			Without surface sterilization	After surface sterilization
	Percent	*Percent*		*Number*	*Percent*	*Percent*
Sept. 2-5	55. 6	60. 2	{Covered and sound	190	3. 2	0. 0
			{Exposed or injured	102	18. 6	. 0
Sept. 13-15	46. 5	58. 3	{Covered and sound	216	17. 1	. 0
			{Exposed or injured	97	52. 6	5 2
Sept. 24-25	33 6	56. 0	{Covered and sound	156	27. 6	. 0
			{Exposed or injured	105	60. 0	8 6
Oct. 5-7	29. 1	50. 0	{Covered and sound	135	33 3	3 0
			{Exposed or injured	66	69 7	9 1
Oct. 15-18	24. 9	40. 2	{Covered and sound	113	40 7	4 1
			{Exposed or injured	60	71 6	28 3

Each ear was broken in the middle with a specially designed apparatus. While the cob was being broken the husk generally remained intact. In the plating room, the husks were opened, short pieces of silk were snipped with sterile instruments from around the central section of the ear, and laid on potato-dextrose agar in Petri dishes (fig. 1, *A*, *d*). Then kernels were taken off with pedicels attached (fig. 2, *A*) and the pedicel was torn off and laid after the corresponding kernel clockwise in the Petri dish, five from each ear (fig. 1, *A*, *a*, *b*). Several fragments from the vascular cylinder were placed in the center of the dish (fig. 1, *A*, *c*). Then the shank was broken off from the ear and a fragment from the center of the butt of the cob was plated (fig. 1, *A*, *e*). A total of 1,240 ears were investigated in this manner.

The broken pieces of ears were immediately dried by forced warm air and later internal seed infection was determined by taking five kernels from the broken part of the ear, neighbors to the kernels that had been plated earlier (fig. 2, *A*). These were then surface-sterilized for 90 minutes in a 4-percent solution of calcium hypochlorite and plated.

METHOD 2

Additional data on the effect of slight earworm damage on kernel infections was obtained in another series of experiments conducted in 1933-37 with five kinds of corn (table 2). Well-matured seed ears with good husk covering were harvested in the usual manner about the middle of October and were rack-dried. Three classifications were made: (1) Ears entirely sound and bright; (2) Ears slightly injured by earworms at the tip only, with no mold growth apparent to the naked eye; and (3) Ears slightly injured by earworms and molded, usually by *Fusarium moniliforme*. Ears with injuries more extensive than those shown in figure 3 or with moldy kernels at other locations than at the tip end were discarded.

The selected ears, 1,462 in all, were tipped and butted by removing 2 or more inches from the tip and about 1 inch from the butt. Care

FIGURE 1.—Unsterilized tissues of corn ears plated aseptically on potato-dextrose agar and incubated for 4 days: *A*, All parts of the ear used in plating, sterile; *B, Fusarium moniliforme* in silks and on parts of kernels that were in contact with silks; *C, F. moniliforme* has penetrated to all parts plated except the butt end of the cob.   *a*, Kernel; *b*, pedicel removed from kernel *a; c*, fragments removed from vascular cylinder of cob; *d*, short sections from silks; *e*, fragment removed from end of cob where shank had been broken off; *f, Monilia sp* Parts shown in *a-d* were taken from nearly the same plane across the center of the ear; all fungus colonies shown except *f* are *F. moniliforme*.
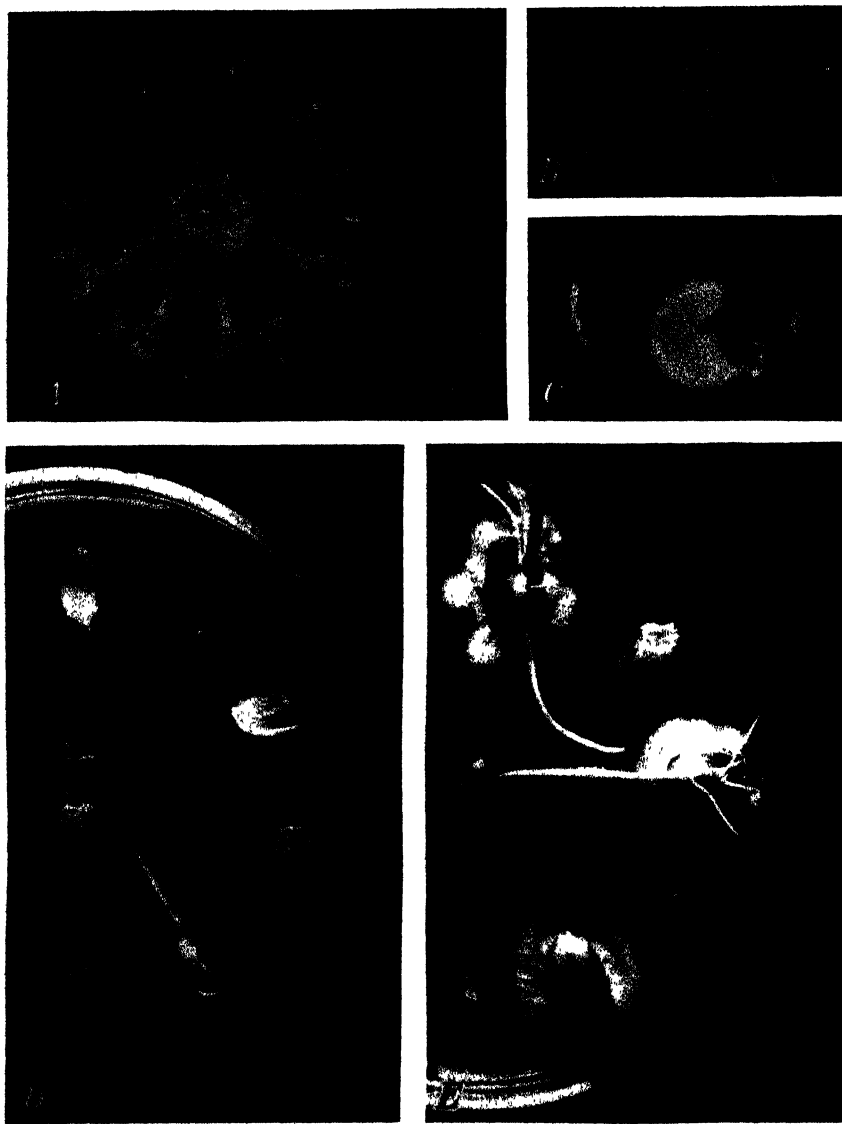
FIGURE 2.— *A*, A broken ear of corn showing how the kernels may be removed from the cob with their pedicels attached; *B*, a colony of *Cephalosporium acremonium* which originated at the point where the kernel had been in contact with the silk; *C*, a colony of *C. acremonium* that originated from a glume; *D*, *C. acremonium* infection in the vascular cylinder and the lower end of pedicels only; *E*, *C. acremonium* infection on kernels only and the distal end of one pedicel. *B* and *C* were photographed from the reverse aside of the agar plates. The progression of infection in *D* and in *E* is in opposite directions.

was taken to go at least 1 inch beyond the worm-injured or moldy area. Four kernels were then removed from each end of the remaining part of each ear, surface-sterilized, and plated on potato-dextrose agar. After 5 days identifications of fungus colonies originating from infections within the kernels were made.

METHOD 3

Mature, well-developed ears of the station strain of Reid Yellow Dent corn were tested for the presence and purity of several kinds

FIGURE 3.—Tip ends of corn ears: *A*, Free from discolorations, worm injury, or moldiness; *B*, slightly damaged by earworms at the tip only; *C*, slightly damaged by earworms, followed by moldiness. These ears are typical of the classes given in table 2.

of fungus infections by removing representative kernels, surface-sterilizing, and plating. From this group two kinds of ears were selected for study: (1) Those that tested pure for one kind of infection but showed no external symptoms on the kernels, and (2) those that tested pure for one kind of infection and showed slight outward discoloration on the kernels from infection.

Five kernels from each ear were then soaked in tap water overnight, sterilized for 1 minute in 1:1,000 HgCl₂, washed in three changes of sterile water, and dissected. After the first section was made (fig. 5, *A*) each part was dissected separately with freshly sterilized instruments to avoid carrying the fungus from one part of the kernel to another.

TABLE 2.—*Ear-tip injury by corn earworms in relation to internal fungus infection of the kernels as determined by surface-sterilizing and plating four kernels from the tip half and four from the butt half of each ear, all the kernels being sound in appearance; studied by method 2 in experiments conducted between 1933 and 1937, inclusive*

Year	Variety or hybrid	Ear-tip condition	Ears tested	Ears with kernel infection at tip or butt half									
				Fusarium moniliforme		Cephalosporium acremonium		Diplodia zeae		Nigrospora species		Gibberella zeae	
				Tip	Butt	Tip	Butt	Tip	Butt	Tip	Butt	Tip	Butt
			Number	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
1933	Reid Yellow Dent	Sound and bright	181	19.9	11.0	2.8	2.8	2.2	13.4	0.0	0.0	0.6	0.0
		Slightly injured, no mold	68	45.6	25.0	.0	1.5	2.9	7.4	0	0	.0	.0
		Slightly injured, moldy	76	78.9	48.7	.0	.0	2.6	7.9	.0	.0	1.3	.0
1935	Golden Beauty	Sound and bright	200	4.5	2.0	1.0	.0	.0	.0	.0	.0	.5	.0
		Slightly injured, no mold	80	12.5	6.3	1.3	2.5	3.0	3.0	1.3	.5	.0	.0
		Slightly injured, moldy	67	37.3	14.9	6.0	3.0	3.0	3.0	3.0	4.5	3.0	.0
1935	Illinois Hybrid 39	Sound and bright	50	6.0	4.0	2.0	4.0	2.0	2.0	.0	.0	4.0	.0
		Slightly injured, no mold	20	12.0	10.0	4.0	4.0	4.0	2.0	.0	.0	9.0	.0
		Slightly injured, moldy	62	60.0	45.0	10.0	5.0	3.2	.0	.0	.0	0	0
1936	Illinois Hybrid 172	Sound and bright	70	17.7	9.7	1.6	.0	3.2	4.8	.0	.0	3.2	.0
		Slightly injured, no mold	39	22.8	12.9	.0	2.9	4.3	2.9	.0	.0	5.7	.0
		Slightly injured, moldy		56.4	33.3	0	0	2.6	2.6	0	0	18.0	.0
1937	Illinois Hybrid 960	Sound and bright	290	2.6	1.7	2.2	1.7	.4	.4	7.0	3.5	.4	.0
		Slightly injured, no mold	187	6.4	3.7	5.9	3.7	1.6	1.1	7.0	5.3	1.6	.0
		Slightly injured, moldy	82	69.5	40.2	2.4	2.4	.0	.0	8.5	8.5	6.1	1.2
	Weighted average	Sound and bright	723	9.0	5.0	2.0	1.7	1.1	4.0	2.2	1.1	1.0	.0
		Slightly injured, no mold	455	16.5	9.4	3.1	3.1	2.2	2.2	3.1	2.2	2.5	.0
		Slightly injured, moldy	284	62.0	35.9	2.8	1.8	1.8	3.2	3.2	3.5	5.3	.3

Kernels that showed white stripes on the surface of the pericarp were studied also by means of free-hand and paraffin-embedded sections.

SPECIES OF FUNGI INVADING CORN EARS

USE OF THE WORD "INFECTION"

For simplicity in presentation, the presence of fungi in any part of the ear described in the first column of table 3 will be referred to as "infection." Strictly speaking, some of this may be only contamination. The fungus entering the ear must obtain food and it is only a question whether the food is obtained from dead or live tissue. The silks are the first parts of an ear that might be considered as dying, but actually as long as the husks are green and tight the unexposed parts of the silks also appear to be alive even though they have already served their function. In healthy ears they are turgid during this period and a bright pale-green, tan, or brownish color, depending on the genetic complex. The fact that only some kinds of fungi were found on or in the silks around the middle of the ear beneath the husks of immature ears makes the propriety of the use of the word "contamination" doubtful in most cases. Not until the husks dry does the silk covered by the husks dry and shrivel, and, except for earworms, it was not until this time that some ears were found with small insects in them, or water beneath the husks.

FUSARIUM MONILIFORME INFECTION

More ears were entered by *Fusarium moniliforme* Sheldon than by any other fungus. This was true even when only ears well covered by husks and free from injury by worms or birds are considered. In the parts of the ear that were well covered by husks, infection was found in or on the silks more often than on the kernels directly beneath the silks (table 3). Next in decreasing order were kernels, pedicels, vascular cylinder, and butt of cob. No doubt infection moved in the direction of decreasing prevalence. With rare exceptions, infection either involved the silks only, or silks plus kernels (fig. 1, *B*), or silks plus kernels plus pedicels, or silks plus kernels plus pedicels plus vascular cylinder (fig. 1, *C*), or silks plus kernels plus pedicels plus vascular cylinder plus butt of cob.

TABLE 3.—*Reid Yellow Dent corn ears infected with Fusarium moniliforme or Cephalosporium acremonium in various locations in the ears, as determined by removing the tissues aseptically and plating on agar medium: Based on 1,240 ears used at Urbana, Ill., in 1933, 1935, and 1937*

[No disinfection used on the parts plated, studied by method 1]

Location of infection in corn ear	Infected with *Fusarium moniliforme*		Infected with *Cephalosporium acremonium*	
	Ears with covered, sound tips	Ears with exposed or injured tips	Ears with covered, sound tips	Ears with exposed or injured tips
	Percent	*Percent*	*Percent*	*Percent*
Silks around middle portion of ear	27.1	54.2	17.3	18.2
Kernels near middle portion of ear	21.8	51.6	16.8	18.0
Pedicels near middle portion of ear	7.9	27.9	9.4	11.2
Vascular cylinder near middle of ear	3.2	12.3	3.0	5.6
Internal tissues at butt of cob	2.3	3.7	2.1	2.2

Three ears were found with infection in the butt of the cob only, 3 with infection in silk and butt of cob, indicating independent areas of infection, and 2 with infections in silk and kernels and butt of cob, also indicating independent infection at the butt. In 1 ear infections were found in butt of cob, vascular cylinder, and pedicels, and in another the same tissues plus kernels were involved. These were the only 2 in 1,240 plated ears where the fungus seems to have come into the kernels by way of the butt of the cob. In 378 ears, on the other hand, the fungus was clearly shown to have come in by way of the tip of the ear.

On September 2–5, about a month after pollination and when kernels were just ready to dent, only 7 percent of the ears well covered by husks were infected with *Fusarium moniliforme*. Kernel infection was even less prevalent, only 3.2 percent of the ears showing surface-borne kernel infection at this time. On succeeding dates, however, the percentage of infected ears increased progressively in considerable amounts (table 1). In ears that had been exposed to the weather by failure of shuck protection at the tip or that had been only slightly injured at the tip end by worms, infections averaged more than twice as many as in the first group mentioned (table 1). Infections with *F. moniliforme* were increased more than any other kind of infection by injuries at the tip of the ear or failure of the husk to protect it. In 1933, when only fully covered ears were used, observations were confined to sound ears and ears with worm-injured tips. In 1937, on the other hand, worm injuries were almost absent, but some of the ears used were incompletely covered by the husks. Both conditions were nearly equal in increasing infections with *F. moniliforme*.

Most of the kernel infection was on the surface of the pericarp. Colonies started at points where the kernel had been in contact with the silks (fig. 1, *B*). In a little later stage the entire pericarp surface carried the fungus (fig. 1, *C*). On September 2–5 none of the infections had penetrated the kernels sufficiently to escape being killed by surface sterilization. The same was true on September 13–15 and September 24–25 for well-covered and sound ears only (table 1). In ears exposed at the tips or slightly injured at the tip only by earworms, penetration by *Fusarium moniliforme* into the kernels at the middle of the ear came earlier and was more extensive. Boewe[2] found more than twice as much *F. moniliforme* rot in ears with open husks as in those with closed husks. The results herein reported, although the data are based on infection in apparently sound kernels instead of in rotted kernels, agree with Boewe's results.

CEPHALOSPORIUM ACREMONIUM INFECTION

Apparently the course of ear infection with *Cephalosporium acremonium* Corda may be by any one of three different routes. In fully half of the infected ears studied by method 1, the course began in the region of the silks. A colony on the surface of a kernel where the kernel had been in contact with the silk is shown in figure 2, *B*. In a large number of other ears, however, the first point of contact of the fungus with the kernel was on the shoulder where the kernel had been in contact with the glume. In some cases the glumes remained attached to the kernels and then the colonies appeared to arise from the glumes. A case in which the glume had become torn and the lower

[2] BOEWE, G. H.   THE RELATION OF EAR ROT PREVALENCE IN ILLINOIS CORN FIELDS TO EAR COVERAGE BY HUSK.   Ill. Nat. Hist. Survey Biol. Notes No. 6.   19 pp.   Urbana, Ill.   1936.   [Mimeographed.]

end turned away from the kernel so that the origin of the colony is clearly shown is illustrated in figure 2, *C*. Under these circumstances, the fungus had, it seems, progressed down the ear by way of the glumes. As pedicels, vascular cylinder, and butt of cob in early cases of such infection were sterile, it is assumed that the fungus must have entered at the tip of the ear.

Less than 2 percent of the ears infected by *Cephalosporium acremonium* had been invaded by way of the butt of the cob. Various stages ranging from infections only in butt of cob to complete penetration through to the kernels were found. A case of incomplete penetration is illustrated in figure 2, *D*. In this case the fungus was found in butt of cob (not illustrated), vascular cylinder (center of dish), and pedicels. The latter, however, were infected only at the end that had been attached to the cob. The ends of the pedicels that had been attached to the kernels, and the kernels themselves, were sterile.

On September 2-5, 9 percent of the ears carried *Cephalosporium acremonium* infection. The fungus was confined to the silks or glumes and surface of the kernels. By September 13-15 prevalence of infection had increased considerably and cases of infection were found in all parts plated. At later dates, increases in infection were found only in the deeper parts, i. e., pedicels, vascular cylinders, and shanks. The prevalence of *C. acremonium* infection was influenced very little by whether the ears were well covered by the husks and were sound or whether they had been exposed at the tips or were slightly injured by earworms (table 3). This is surprising in view of the fact that the vast majority of the ears appeared to have been invaded by the fungus by way of the tip of the ear.

In other experiments (method 2) presented in table 2 there was stronger evidence that prevalence of *Cephalosporium acremonium* infection within the kernels was increased by injuries by worms or exposure at the tips of the ears.

GIBBERELLA ZEAE INFECTION

Only 9 ears of the 1,240 plated by method 1 showed infection with *Gibberella zeae* (Schw.) Petch. All these were found in 1935, and in every case the fungus had entered by way of the tip of the ear. Colonies on kernels originated somewhere on the crown half, indicating contact with silks. No cases of infection were found in pedicels, vascular cylinder, or butt of cob. It should be recalled that the ears were broken in the middle only, and that in all this work the ears used were sound in appearance except for occasional slight amounts of mold on the tip end. Thus the fungus was halfway down the ear before it caused visible rot. This was a greater distance than was usually found with *Diplodia zeae* in similar immature ears. Ears studied by method 2 showed a somewhat higher percentage of *G. zeae*-infected ears. Again, all the infection apparently originated by way of the tip end of the ear (table 2). Ears found with infections on the butt half were infected more extensively on the tip half. In other studies, however, the writer has seen a few ears with gibberella rot apparently starting at the butt.

NIGROSPORA INFECTION

Nigrospora oryzae (B. and Br.) Petch and *N. sphaerica* (Sacc.) Mason were both present in the material plated, but whether there

are valid reasons for separating this fungus into two species is still an open question. The term *Nigrospora* spp. is used here to cover both species. The earliest infections observed by method 1 were September 13–15, when 4 infected ears were found. One carried infection in the silks only, 1 on silks and kernels only, 1 at butt of cob only, and 1 in butt of cob and vascular cylinder only. Contrary to expectation, prevalence did not increase much with later platings. In 10 out of a total of 17 *Nigrospora*-infected ears found, infection appeared to have started at the butt of the ear; in 7 at the tip of the ear. Internal kernel infection occurred in only 2 of these 17 ears, and only in the October harvestings. As concluded from these figures and from the data in tables 2 and 4, *Nigrospora* may enter the ear at both the tip and butt ends, the latter being most common.

DIPLODIA ZEAE INFECTION

Infection with *Diplodia zeae* (Schw.) Lév. was very prevalent in 1933. Although only externally sound appearing ears were brought to the laboratory, when the husks were opened, 83 ears were found to be already visibly overrun by the diplodia fungus over a small to large part of the ear. These ears were not plated in the usual way by method 1 and are not included in the total count of 1,240 ears mentioned earlier. In addition, 28 sound-appearing ears, when plated in the manner described for method 1, also developed diplodia colonies. Seventeen of these ears were infected at the butt of cob only; 5 showed progressive stages beginning at the butt and involving vascular cylinder and sometimes pedicels and kernels. Six showed that the fungus had entered by way of the tip of the ear.

Pieces from the ears that were visibly partly overrun by the diplodia fungus were also plated but in a different manner. Sound-appearing kernels, pedicels, fragments from the vascular cylinder, and a piece from the center of the pith of the cob were removed and plated at a distance of 1 inch away from visible fungus development and also at a distance of 2 inches. After plating, 60 percent of the ears showed infection of the kernels at the 1-inch distance, 40 percent at the 2-inch distance. Infection showed most often in the kernels, least often in the pith. Thus in approximately 60 percent of the diplodia-infected ears, the invisible advance of the fungus was less than 2 inches ahead of the visible area of infection as viewed on the surface of the ear. These were early season infections. In later infections when ear development is more nearly mature, the situation is probably somewhat different, for in the latter case one frequently finds ears with the germs of all the kernels darkened by diplodia attack without evidence of this damage on the surface of the unshelled ears.

MONILIA INFECTION

A species of *Monilia* was a very common source of infection in corn ears in each year of the investigation. When the tissues were plated on potato-dextrose agar the colonies were a light-pink color, very much like *Fusarium moniliforme* or *Cephalosporium acremonium*, but more restricted in colony size and more delicate in texture. A colony is shown in figure 1, *B, f.* The spore sizes were 2.5 to 3 by 5 to 10 microns. In 1933 *Monilia* was not observed on the plates until September 25, but in 1935 in the first plating, September 4, it was the most prevalent fungus, being shown by 31 ears as compared with

25 showing *F. moniliforme*. This lead was maintained throughout the season. In 1937 *Monilia* was present to about the same extent as *F. moniliforme* in well-covered sound ears and was observed in the first tests of the season. Failure of husks to cover the ears did not aggravate infections by *Monilia*, and thus in exposed ears *F. moniliforme* was more prevalent. Because of the small size of the colonies, some of them were doubtless covered by other fungi and so a full record was not obtained.

The course of infection with this *Monilia* sp. was very much like that of *Fusarium moniliforme*, except that prevalence was not increased significantly by corn earworm injury or failure of complete husk protection. Entry into the ear was apparently exclusively in the region of the silks. The pedicels and vascular cylinder were readily invaded, and a small percentage of ears showed complete penetration through to the butt of the cob. Internal seed infection, however, was not found.

PENICILLIUM INFECTION

The plates were fairly free from penicillium when the ears were tested in September. In October when the husks became dry and somewhat loose, penicillium colonies were more abundant, especially in ears with incomplete husk covering or those with earworm damage at the tips. Almost all of this infection was confined to the silks and kernels. The prevalence of penicillium was highest in the October 16, 1935, plating, when the following percentages were recorded: Silks 37, kernels 33, pedicels, 1.5, vascular cylinder 1.5, butt of cob 3.0. A number of species were involved and no attempt at identification was made except that watch was kept for *Penicillium oxalicum*. This species causes a seedling blight of corn, and is identified comparatively easily. In 1933, of 28 ears found infected with penicillium, 18 were identified as *P. oxalicum*. But in 1935 and 1937 this species was not observed.

Extremely few cases of penicillium infection within the kernels were found in these experiments. In another experiment, however, in which mature ear corn, picked as seed ears, was dried slowly, considerable internal seed infection with penicillium was found (5).[3] According to the writer's observations in Illinois, the penicillia rarely penetrate into normal uninjured corn kernels as long as they are in an actively growing condition, and if the ears are dried rapidly as soon as they are mature very little internal infection is found.

CORN EARWORM INJURY AND FUNGUS INFECTION

Some aspects of the relation of slight earworm injury and incomplete husk protection at the tip of the ears to fungus infection at the middle of the ears have already been discussed under the different fungus name headings, and some of the data have been given in table 1. Another experiment dealing specifically with earworm damage was conducted as explained under method 2, and the results are given in table 2. It should be emphasized that these experiments were confined to ears with worm injuries only at the tip. They were not concerned with the identity of fungi at the injured location, but rather

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[3] Italic numbers in parentheses refer to Literature Cited, p. 441.

with internal infection in kernels at some distance away from the injury.

It is already well known that corn earworm damage is followed by increased corn ear rot by *Fusarium moniliforme*. In the present work, based on kernel infection rather than rot, *F. moniliforme* infections were similarly increased most by earworm damage. Next in order were infections by *Gibberella zeae*, *Nigrospora* spp., and *Cephalosporium acremonium*. Infection with the last two was only slightly influenced by earworm injury. Infection with *Diplodia zeae* was apparently not at all affected by this factor.

Ears with worm injuries that had become moldy (figure 3, *C*) had higher percentages of kernel infection, on the average, than worm-injured ears that were free from mold. In general, it appears safe to assume that the injuries that became moldy occurred earlier, when the ears were more immature and more susceptible to infection, than injuries that did not become moldy.

INFECTION AT BUTT OF COB IN RELATION TO COB DISCOLORATION

As the season advances, fungi can be isolated in progressively greater numbers from the internal tissues where the shank breaks off from the cob and discolorations of these tissues become more pronounced. This situation was especially evident in 1933. On October 23 and 24, 546 apparently sound ears were harvested with the shanks intact, internal tissues from the butt of the cob were plated as explained under method 1, and the ears were classified into 4 groups according to the appearance of the butt of the cob (fig. 4). All ears were well covered by the husks and only those that appeared entirely sound after the husks had been removed were used (fig. 3, *A*).

The term "slightly discolored," applied to butts of cobs, refers to light-yellow, drab, tan, or pink colors, while "badly discolored" refers to strong drab, tan, brown, pink, or red colors. Nearly all of the cobs had a natural pink, red, or brown color of the internal vascular cylinder, even those classified as "white" at the butt. Usually this natural color did not extend down to the place where the shank broke off, but occasionally no doubt it did so. When this happened it was difficult to distinguish between natural color and discoloration caused by infection. In corn genetically pure for white internal cob color, much better separations could be made.

In spite of the fact that some ears probably carried natural genetic colors which were classed as "discolored," the relationship between discolorations and the presence of fungus infection in the interior tissues at the butt was outstanding (table 4). The correlation between extent of butt of cob discolorations and infections by *Diplodia zeae*, *Nigrospora* spp. and *Fusarium moniliforme* was especially good (table 4). Infections with *Cephalosporium acremonium*, *Gibberella zeae*, and *Penicillium* spp. bore little or no relationship to shank discoloration. *Nigrospora* infection was strongly related to shredded shanks.

More ears were infected with *Diplodia zeae* and *Nigrospora* spp. at the butt of the cob than in the kernels. This fact, with other evidence discussed earlier in this paper, leaves no doubt that most of the ear infections with these organisms came in through the butt of the cob.

There were, however, exceptions with *Nigrospora*, and many exceptions with *Diplodia*. With *Fusarium moniliforme*, on the other hand,
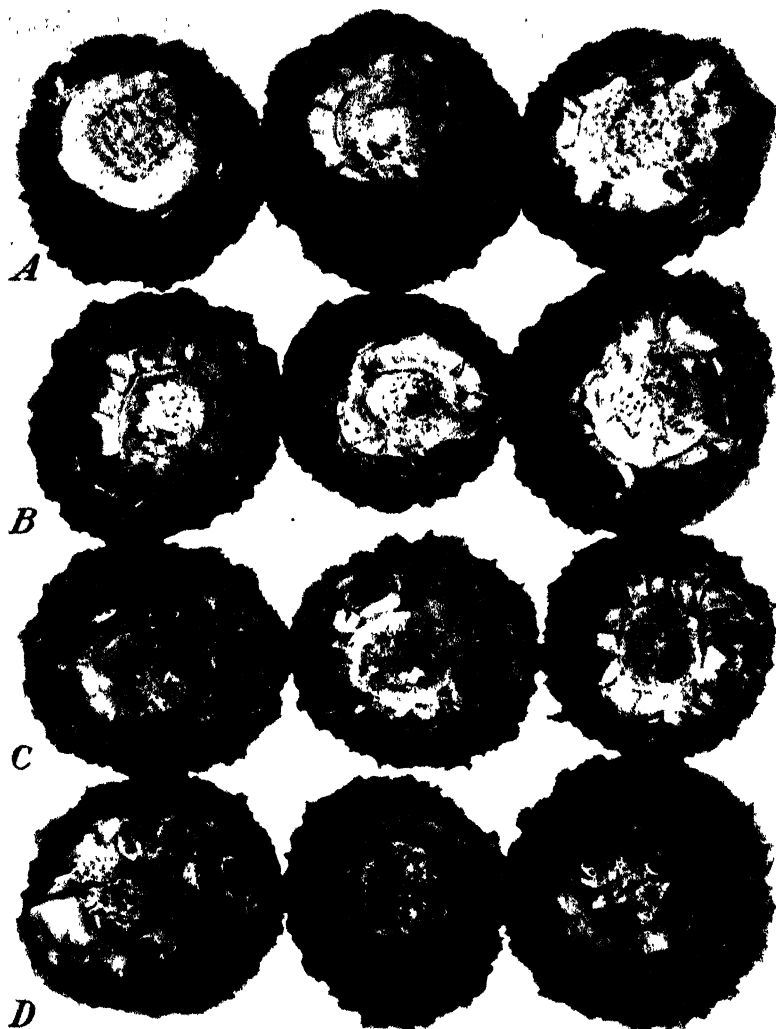
FIGURE 4.—Butts of cobs: *A*, Bright, sound, healthy appearance; *B*, slightly discolored; *C*, badly discolored; *D*, discolored and pith disorganized so that the vascular strands appear shredded. These butts are typical of the classes in table 4.

kernel infection was more prevalent than butt of cob infection. This corroborates the results obtained by method 1, namely, that kernel infections with *F. moniliforme* nearly always precede butt of cob infections.

TABLE 4.— *Ears of Reid Yellow Dent corn showing fungus infection when tested a butt of cob and in kernels around central part of ear after having been classified according to extent of discoloration at the butt of cob, Urbana, Ill., 1933*

Kind of ears and fungus isolated	Ears infected when cob at butt was—			
	White (141 ears)	Slightly discolored (185 ears)	Badly discolored (130 ears)	Discolored and shredded (90 ears)
	Percent	*Percent*	*Percent*	*Percent*
Ears with butt of cob infection				
None	79.4	60.0	34.6	15.6
Diplodia zeae	2.8	14.1	28.5	34.4
Nigrospora spp_	2.8	7.6	6.1	23.3
Fusarium moniliforme	5.0	7.6	17.7	17.8
Cephalosporium acremonium	0	.5	.0	0
Gibberella zeae	0	1.6	.0	0
Penicillium spp	5.7	4.3	8.5	6.7
Miscellaneous and unidentified	4.3	4.3	4.6	2.2
Ears with kernel infection [1]				
None	65.2	55.1	42.3	25.5
Diplodia zeae	4.3	11.9	21.6	27.8
Nigrospora spp	0	0	.0	11.1
Fusarium moniliforme	27.7	31.4	35.4	38.9
Cephalosporium acremonium	2.1	2.7	3.1	2.2
Gibberella zeae	7	0	8	0
Penicillium spp	7	1.6	0	1.1
Miscellaneous and unidentified	1.4	4.3	3.1	4.4

[1] Recorded as an infected ear when 1 or more of the 8 kernels tested per ear by surface-sterilizing and plating showed infection. Some ears showed the presence of more than 1 kind of fungus, and each was recorded

POSITION OF INTERNAL SEED INFECTION

The path of infection to the kernel having been followed, it is desirable to know where the fungi enter the kernel and which tissues are most readily invaded. Manns and Adams (7) found that in mature, sound-appearing kernels the mycelium of *Fusarium* and *Cephalosporium* often occurred in the tip cap (fig. 5, *a*) and especially in the cavity between the tip cap and the brown to black layer covering the scutellum (fig. 5, *e*). They state: "The development of the fungus from the cap into the germ and endosperm appears to be restricted by the black layer." In some cases, however, they found fungus mycelium also in the cavity around the radicle and plumule.

Johann (4) called the dark layer, mentioned above, the "closing layer," and found that it was rather impervious to fungus penetration once it had formed, but in the earlier stages of kernel development this protection was lacking.

Branstetter (1) cut surface-sterilized, sound-appearing kernels into five pieces. When infection occurred it was always present in the section nearest the tip end, and in 31 percent of the kernels infection occurred only in this area.

An experiment was set up to determine in which particular tissues of the kernels infection occurs. The technique is described under method 3. Ears from the 1938 crop on the station farm were used.

Infection was found to be most common in the tip cap. This agrees with the results of other investigators. Next in decreasing order of infection prevalence were tissues of the germ, floury endosperm, and horny endosperm (fig. 5). This was true of all the fungi studied (table 5). When infected ears showing no visible symptoms on the
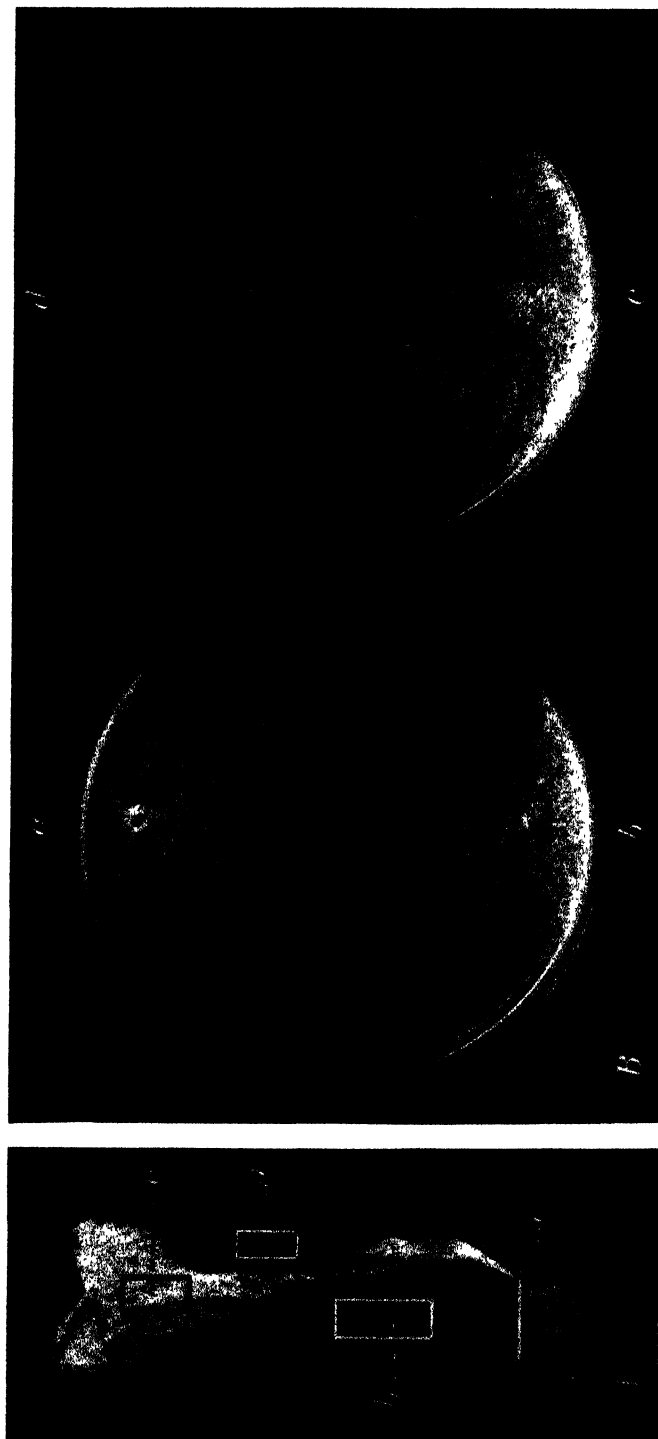
FIGURE 5.—*A*, Parts of the corn kernel used for plating to determine infection: *a*, Tip cap, cut off so as to include the dark layer and hollow space at *e*; *b*, central part of germ; *c*, floury endosperm *d*, horny endosperm. *B*, The letters correspond to the same letters in *A*, showing, in this case, *Diplodia zeae* colonies arising from all the tissues plated except the horny endosperm.

TABLE 5.—*Fungus infection in different parts of well-developed mature Reid Yellow Dent corn kernels studied by method 3; fragments dissected out under aseptic conditions and plated on agar medium: Class 1, kernels showing no outward signs of infection; class 2, kernels slightly discolored by infection; Urbana, Ill., 1938*

Class	Cause of infection	Ears used	Kernels plated	Infection in tip cap	Infection in germ	Infection in floury endosperm	Infection in horny endosperm
		Number	*Number*	*Percent*	*Percent*	*Percent*	*Percent*
1	*Diplodia zeae*	6	30	96 7	43. 3	20 0	6 7
	Fusarium moniliforme	11	55	76 4	21 8	1 8	.0
	Nigrospora spp	5	25	48. 0	8 0	4 0	.0
	Cephalosporium acremonium.	4	20	80 0	.0	.0	.0
2	*Diplodia zeae*	19	95	100 0	98 9	82 1	28 4
	Fusarium moniliforme	33	165	99. 4	83 0	70 3	48. 5
	Nigrospora spp	10	50	100 0	24 0	12. 0	4 0
	Cephalosporium acremonium	2	10	100. 0	40 0	30. 0	0
	Penicillium spp	4	20	100. 0	95. 0	60. 0	35 0

kernel were selected, not all kernels on the ear carried infection, but when infected ears having kernels with slight discolorations were selected, infection was practically 100 percent (classes 1 and 2 respectively, table 5). *Cephalosporium acremonium* in class 1 showed least ability to spread into other parts of the kernel from the tip cap. In class 2, both *C. acremonium* and *Nigrospora* spp. made the least extensive growth into various parts of the corn kernels. Discolorations from *C. acremonium* consisted of white streaks (fig. 6, *B*). In the writer's material, this fungus did not cause significant kernel rot or discolorations beyond the streaking just mentioned. With regard to *Nigrospora*, it should be borne in mind that the kernels used were well developed and had a well-differentiated horny endosperm. Thus these results do not apply to infections that arrest the development of kernels so that the horny endosperm is poorly developed. In the latter case the germ becomes badly disintegrated (*2*).

Another tissue plated from these kernels was the pericarp from a considerable area of the middle of the back of each kernel. After soaking overnight, the pericarp can be pulled away easily, the break occurring near the testa (*4*). By the method used, the pericarp usually plated sterile. It was discovered later that the sterile pericarp platings were due not always to absence of infection but to penetration of the disinfectant used for surface sterilization.

NATURE OF WHITE STREAKS ON PERICARP

A special study was made of the nature of the white streaks often found on the pericarp of infected kernels (fig. 6). That these streaks may take form rather late in the development of the kernel was shown in 1938 when corn was lodged artificially on September 16, about 15 days after denting had been completed and when the moisture content of the kernels was approximately 35 percent. When harvested, kernels with streaked seed coats were numerous in the lodged corn, but rare to absent in the standing corn. This type of streaking occurring in the field may be of a different nature from that developing when corn is being germinated in a germinator. The latter has been mentioned by Manns and Adams (*7, p. 504*), who state: "As a rule, the fungus ramifies between the pericarp and epidermis, resulting in pronounced discoloration of the pericarp and involving the lower half

of the kernel. Often the discoloration will appear as irregular streaks, extending from the germ end toward the crown."

Streaked kernels developing in the field invariably showed infection when surface-sterilized whole kernels or tip ends of the kernels were plated. Fungi isolated were *Fusarium moniliforme, Cephalosporium acremonium,* and *Nigrospora* spp. Streaks extending up to the top of the crown were most often associated with *F. moniliforme. Nigrospora* usually caused wider streaks than the others and these had a tendency to coalesce near the tips of the kernels. Streaks caused by *C. acremonium* most often were limited to the lower half of the kernels, and had a tendency to coalesce at the tips. Figure 6, however, also shows some exceptions to these statements. At times the streaks

FIGURE 6.— Kernels with white streaks on the pericarp caused by infection with: *A, Fusarium moniliforme; B, Cephalosporium acremonium; C, Nigrospora* spp. Identified by surface-sterilizing and plating.

were distinctive enough to permit one to identify the infection with a fair degree of accuracy. Durrell (*2, fig. 4*) illustrated streaks caused by *Basisporium gallarum* (*Nigrospora* sp.), and Edwards (*3, fig. 20*) showed streaks caused by *Gibberella fujikuroi* var. *subglutinans* (=*F. moniliforme* var. *subglutinans*).

By examination with a high-power binocular microscope, fissures in the pericarp surface coinciding with the white streaks can be seen, although this surface appears smooth and intact to the naked eye. Free-hand sections were made of mature kernels with each of the three kinds of infection mentioned above. Pieces of kernels, after being disinfected, were soaked overnight in water, cut, and stained with Pianese III B. This not only demonstrated broken-down cells in the pericarp but also gave good differentiation of fungus hyphae in red as contrasted to the pericarp cells in green. Pieces of pericarp cleared in chloral hydrate showed the cells to be very pitted and very long. This latter fact seems to be the reason for the linear direction of the streaks. Additional sections were cut also from pieces taken from the back of streaked *Fusarium moniliforme*-infected kernels

which had been killed in Gibson's fixative, embedded in paraffin, and stained in gentian violet and Pianese.

The streaks were definitely not caused by the mycelium growing between the pericarp and testa and thus lifting the colorless pericarp away from the yellow layers beneath. What actually happened was that the fungus caused a break-down of the pericarp cells, often at the surface (pl. 1, *B*, *C*) but sometimes in the middle area (*F*) and sometimes next to the testa (*D*). The normal cells are very thick-walled and possess considerable transparency. After corrosion by infection, only shriveled membranes remain and these areas have lost their transparency and have taken on a chalky appearance.

Infection of the pericarp undoubtedly takes place before complete maturity and appears to be effected in two ways: (1) Internally at the junction with the tip cap, and (2) externally by action of the fungus on the surface. Once a cell has been entered, progression is linear because the cells are very long. Progression at right angles takes place slowly. The mycelium can clearly be seen in the lumen of the cells and in the pits. However, passage from cell to cell through the pits does not seem to occur readily; spread sidewise is accompanied by considerable disintegration of the cell walls.

DISCUSSION

In corn ears that were well protected by the husks and had not been entered by earworms, a surprisingly large number were found to be sterile until practically mature. As a 3-year average for the dates September 2–5, 13–15, 24–25, October 5–7, and 15–18 the corresponding percentages of sterile ears were 77.6, 68.2, 45.3, 46.4, and 48.0. It would appear as though a stationary point were reached about September 24–25, but this is not true with respect to the whole population because the percentage of ears well covered by husks and free from earworm attack is steadily decreasing during this period. Perhaps the plugging of the channel through the husks at the end of the ear by the silks is a help, and it is highly probable that, prior to complete maturity, the tissues involved along the path of infection are a poor medium for the growth of saprophytic fungi.

Nearly all fungi in corn ears were found to enter either by way of the channel through the husks at the tip of the ear or at the base of some of the husks and thence through the shank. One way in which infection may reach the shank after traversing the length of the husk has previously been illustrated (*6*, fig. 27). A local shank infection which resulted in entry into the ear is shown in figure 7, *A*. Sometimes, however, the ear becomes infected first and the shank second. The appearance of a rot at the butt end of the ear does not necessarily mean that the infection entered at the butt. In experiments with artificial inoculations (unpublished) it was observed that, after the ears had dented, some of the inoculations made with *Diplodia zeae* at the tip of the ear resulted in infections like that in figure 7, *B*. The fungus apparently made its way down the ear without prominent visible signs, and produced rot at the lower end where moisture was highest.

Occasionally *Diplodia* may progress from an infection in the stalk through the shank and cause ear rot. This was proved by making stalk inoculations in the internode beneath the node that bears the
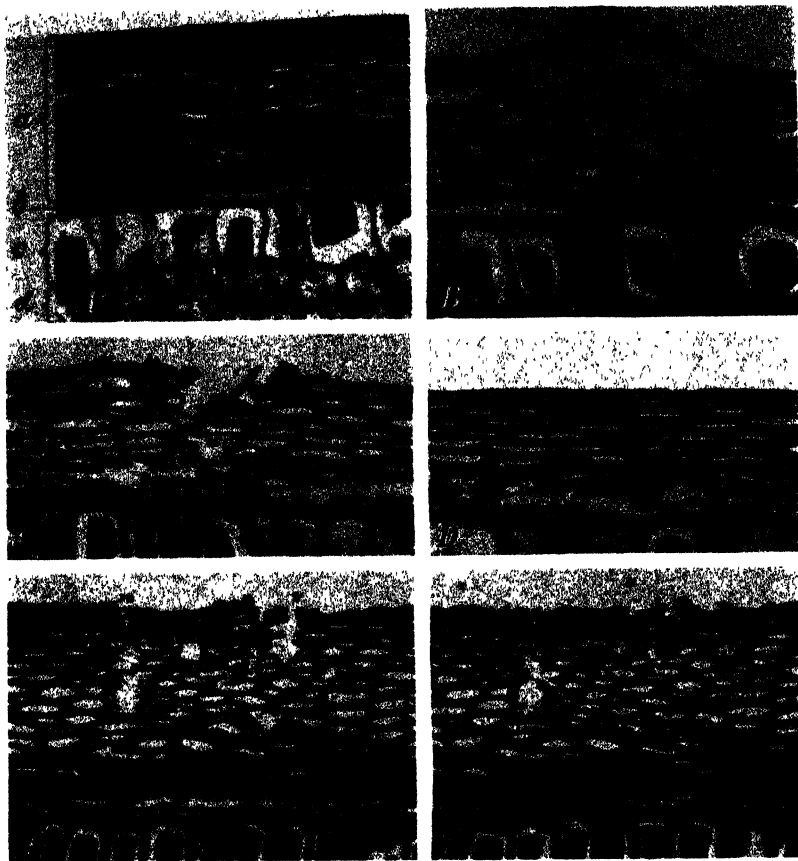
A, Section through the pericarp of a sound nouinfected kernel of corn from the side opposite to the germ and cut at right angles to the long axis of the kernel: a, pericarp; b, testa; c, aleurone; d, horny endosperm.

B-F, Sections through white streaks of *Fusarium moniliforme*-infected kernels similar to those shown in figure 6: B, Tissues severely broken down in the outer half of the pericarp; C, disorganization, starting at the surface, has progressed through the entire thickness of the pericarp; D, serial section from the same kernel as in C but at some distance from it, showing disorganization only near the testa; E, disorganization of cells resulting in long internal channels; F, serial section from the same kernel as in E. Some of the sections, especially E and F, show the dry dormant mycelium. × 265.

shank. A small but significant increase in diplodia ear rot was obtained in this way.

It was supposed at one time that ear infection with *Cephalosporium acremonium* was the result of systemic infection of the plant (8).
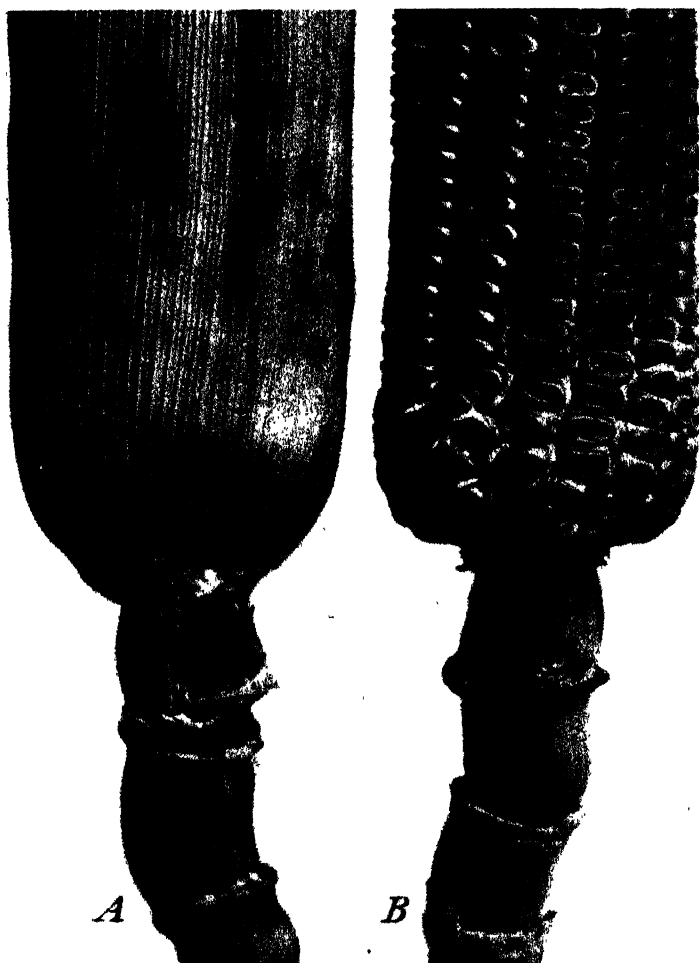
FIGURE 7.—Corn ears rotted at the butt end by *Diplodia zeae: a,* Infection started at the base of husks; *b*, infection started at the tip of the ear. This ear was artificially inoculated at the tip end, but rot developed primarily at the butt where moisture was highest.

In the experimental work herein reported, this was not true in a large majority of the cases. There were, however, some clear-cut cases (fig. 2, *D*) of *C. acremonium* infection that came via the butt of the ear. Whether or not these cases were the result of systemic infection was not determined.

Having made contact with the ear at the tip end, some fungi ramify pretty much to all parts of the ear before kernel penetration takes place, while in very immature ears some others may cause penetration at a distance of only a few inches behind the front ad-

vance of the mycelium. Environmental conditions, variety of corn, and stage of development of the ears are important factors determining ability of fungi to overrun ears and penetrate into kernels. In general, if one were to classify the fungi entering at the tip of the ears according to their relative ability to penetrate into kernels so that the fungus persists after surface sterilization, the ranking, according to information at hand, in descending order would be: *Diplodia zeae, Gibberella zeae, Nigrospora* spp., *Fusarium moniliforme, Cephalosporium acremonium,* and *Pencillium* spp. *Nigrospora* spp. occupy an uncertain place in this ranking because, in general, they become parasitic only under certain host responses to the environment. If the ranking were according to ability to cause rot, *C. acremonium* would come last. This ranking, of course, is irrespective of prevalence.

Entry into very young kernels may apparently be effected anywhere through the pericarp and testa by *Diplodia zeae* and *Gibberella zeae,* but as the kernels develop, the testa offers increasing resistance (*4*) and finally even these fungi are limited to entry via the tip cap. If any of the other fungi mentioned in this paper ever enter into the internal tissues enclosed by the testa of undamaged corn kernels in any way except via the tip cap, the occurrence seems to be rare. However, when mechanical damage or growth cracks have occurred while the kernels are still juicy, *Fusarium moniliforme, Pencillium* spp., and other fungi have been found to enter at the injured place.

When entry is via the tip cap, after denting has occurred, the fungus may pass up into the pericarp and also into the internal tissues, but apparently it cannot pass through the testa from one to the other. Pericarp infection can usually be diagnosed by the presence of white, pink, or dark streaks. It is found most often when infected ears dry or mature very slowly as, for instance, in lodged corn when the outer husks are in contact with the ground or in standing corn when protracted wet weather occurs during the final maturing period. Infection in the pericarp is easily killed by ordinary surface disinfection, but infection in the tip cap and internal parts persists. After entering into the interior of the kernel, *Diplodia* ramifies most easily through the aleurone, according to Johann (*4*). In the present work, *Diplodia zeae, Fusarium moniliforme, Cephalosporium acremonium,* and *Nigrospora* spp. were all found more often in the germ than in the soft or horny endosperm. This agrees also with the appearance of infected kernels when cut open; i. e., the germ is the area that is most often discolored.

SUMMARY

The method of experimentation consisted of plating on agar medium natural tissues, unsterilized, from selected parts of corn ears in various stages of development; the plating of mature surface-sterilized whole and dissected corn kernels; and the preparation of histological sections of corn kernels.

Fusarium moniliforme was the most prevalent fungus in corn ears. In nearly all cases the fungus entered in the region of the silks, the kernels became contaminated at points of contact with the silks; thence infection spread to the pedicels, vascular cylinder, and finally the shank. In very few cases did infection proceed in the opposite manner, from shank to kernel. Internal kernel infection in sound-

appearing kernels did not become established until the ears were approaching maturity.

A large percentage of infection with *Cephalosporium acremonium* also took place in the manner just mentioned. Another common method of infection was of a type in which the fungus made contact with the lower half of the kernel surface. Apparently the fungus progressed down the ear in the area in which the glumes are located. A smaller percentage reached the kernels by way of the butt of cob, vascular cylinder, and pedicels. Internal kernel infection became established when the ears were approaching maturity.

Gibberella zeae infection practically all started at the tip ends of the ears and progressed down the ear most rapidly in the region of the silks.

Diplodia zeae and *Nigrospora* spp. caused infection by entering at both the tip and butt ends of ears with a slight majority in favor of the latter. Fungus penetration at the butt was very largely the result of local infections on the shank. *D. zeae* caused a more active and more generalized rot in the wake of the advancing mycelium than any of the other fungi.

A species of *Monilia* was very prevalent in some seasons. It entered in the region of the silks and progressed over the surfaces of the kernels and invaded the pedicels and vascular cylinder. Internal kernel infection, however, failed to take place.

Penicillium infections behaved very much like *Fusarium monili-forme* infections except that first appearances were later in the season, prevalence was lower, and internal kernel penetration into sound-appearing kernels was practically absent.

Exposure of the tip of the ear from incomplete husk protection or slight injury at the tip from earworms caused marked increases in infection by some fungi.

Discoloration at the butt of the cob where the shank was broken off was found to be a strong indication of infection at the butt and also of the kernels of ears that had previously been selected for good husk covering and freedom from worm damage.

White streaks on the pericarp of kernels were found to be caused by the invasion of the pericarp by *Fusarium moniliforme, Cephalosporium acremonium,* or *Nigrospora* spp. The white color was due to the disintegration of cells which caused them to lose their transparency and take on a chalky appearance.

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STUDIES ON THE VIROPLASM HYPOTHESIS[1]

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INTRODUCTION

Fifteen years ago it was suggested from this laboratory that some part of the normal protoplasm or cytoplasm of one species, if properly introduced into the cells of another species, might find conditions compatible for growth in that species, and bring about the abnormality which is known as virus disease (6).[2] This "viroplasm" hypothesis[3] was based largely on results secured with extracts from apparently healthy potato plants inoculated into other members of the Solanaceae. Subsequent evidence has shown that practically all standard varieties of American potatoes are symptomless carriers of the mottle virus or ring spot virus in question, and that the hypothesis was untenable. Other evidence would be required to support the viroplasm hypothesis if it were to remain even a probable theory. Not many investigators have given this theory of the origin of viruses serious second consideration, but its possibilities have appealed to some pathologists who have encouraged further efforts to determine its validity.

It must be assumed that the possible compatible combinations under the viroplasm hypothesis are relatively rare, otherwise a legion of different viruses in epidemic proportions would be expected. If such combinations are as rare for example as chimeras, compatible interspecies grafts, or natural crosses between species, the number of viruses actually originating spontaneously in nature through transmission of foreign protoplasm by certain insect species or other natural means might be expected to be roughly proportional to the number of natural viruses known to exist. The possible number of compatible combinations of the "viroplasm" type which may be attempted is, of course, enormous, even if it were limited only to related species, e. g., within a family. The introduction of still other circumstances, such as the possible variations in artificial methods of transfer of protoplasm or the probability of the labile forms of protoplasm (attenuated forms) requiring repeated culturing or transfer in new hosts in order to secure the full expression of symptoms are of such a nature as to discourage further experimental efforts in this direction.

Even when limiting the trials to only family, as was done in the investigation to be reported, the result secured soon become so intricate and involved that it is necessary to repeatedly eliminate interesting possibilities from further trial. That unusual compatibility of a similar nature may exist between widely different plant families as well is suggested by Daniel's (2) combinations in grafts of such plants as sunflower and melon, cabbage and tomato, maple and

lilac. If such unions are to be taken as any indication of the possibilities of the existence of viroplasm, the chances of securing a compatible combination by trial and error are extremely small.

The experiments presented in the present paper yielded no positive proof in support of the validity of the viroplasm theory. The efforts are significant rather as demonstrating the problems to be expected in further studies of this type. Nevertheless, the results obtained are of some interest and value since they have brought to light two hitherto apparently unknown viruses, as well as what appears to be an allergic response in plants to the metabolic products of another plant species.

An abstract of this paper was published earlier (7).

EXPERIMENTAL METHODS

The Leguminoseae were chosen originally for the purposes of this study chiefly because many genera and species were obtainable from collectors of this plant family [4] and because this group is known to be affected by a considerable number of viruses. Many species of this family are not suitable for test plants either because of their relatively slow growth or because of leaf size or shape. However, the number of possibilities of cross inoculation are still great, even though only a few species are chosen to be inoculated with extracts from the others. The common bean (*Phaseolus vulgaris* L.) is an ideal test plant and consequently most of the inoculations were made to varieties of this species. The variety Stringless Green Refugee was always used unless otherwise stated. Fresh extract from apparently healthy species of legumes was transferred by the wiping method (now often used in virus studies) to the primary leaves of young healthy bean plants. Carborundum powder was regularly used with the inoculum in order to increase the probabilities of transfer, although its use was later shown to be often unnecessary.

The use of inoculum from young plants produced from seed has a distinct advantage over the use of inoculum from vegetatively propagated plants like the potato, since it is not at all likely that all individual plants are symptomless carriers of a virus as in the case of the potato (*6*). Occasional seed transmission of known viruses in legumes is not unlikely, and special precautions should of course be taken to avoid confusion through such a possibility. Beans used as test plants were obtained from "mosaic-free stocks" and rogued further for any signs of the disease before being used for test plants. It may be stated here that no instance of the seed-transmission of a previously described virus was noted in the trials except that of ordinary bean mosaic virus.

The plants were grown, and all inoculations made, in a greenhouse at a temperature averaging about 80° F. The plants to be inoculated were grown in 4-inch pots, with five beans planted in each pot. All small or abnormal seedlings were weeded out, usually leaving only three or four plants to the pot. Two pots of test plants (seven or eight plants) were commonly inoculated with each extract tested. Records were taken on all inoculated plants from 5 to 10 times at intervals of 1 to 3 days, always comparing them with uninoculated plants of the same age or other forms of control plants.

[4] The writer is indebted to the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and to many individuals for furnishing seeds of the species used.

The physical properties of the viruses isolated were tested in the same manner as previously described from this laboratory.

EXPERIMENTAL RESULTS

One hundred and twenty-two species of legumes representing 50 genera were grown and inoculated to the bean and certain other test plants. It seems unnecessary to list these genera or species in the present connection, or to present in detail the large number of negative results secured from the inoculations. With some species only a single inoculation trial was made, whereas with others up to 20 and, in one instance, over 100 separate series of inoculations were made from a single species to over 700 test plants. It is logical to assume that some known or unknown viruses might be carried through the seed of certain species and that the species involved might act as a symptomless carrier. One hundred percent seed transmission of a previously existing virus would not be expected, although 100 percent of compatibility might be expected or assumed to be necessary for proof on the basis of the viroplasm hypothesis. Repeated trials, in which single healthy plants were used as a source of inoculum, became especially desirable where any viruslike symptoms had been expressed in any prior trials between two species. If apparently healthy individual plants of a single species continued to yield responses on the inoculated host, the results naturally became most suggestive of those to be expected or assumed on the basis of the viroplasm hypothesis. Such an ideal result is obviously not necessary to the hypothesis. Quite as likely, positive results may be expected only occasionally, just as is the case of grafting or hybridization between species with a low degree of compatibility.

AN ALLERGIC REACTION IN PLANTS

When extracted juice of the Tangier pea (*Lathyrus tingitanus* L.) is wiped on the young primary leaves of the bean (*Phaseolus vulgaris* L.), the developing new secondary leaves become mildly chlorotic, veincleared, and somewhat stunted in growth as compared to control plants (figs. 1, 2). Although the symptoms may be modified to some degree by environment, they are retained throughout the life of the plant. The symptoms are so similar to those which may be expected in a relatively mild virus disease that experiments were continued with this material for several weeks in the belief that a virus was concerned. On this assumption, separate inoculations were made to the bean with over 100 individual Tangier pea plants (fig. 3) grown from seed secured from three sources. Typical symptoms were always obtained, suggesting either that all Tangier peas were symptomless carriers of a virus transmitted 100 percent through the seed or that the results supported the viroplasm theory. During the course of these trials, efforts were also directed toward serial transmission of the reaction and studies on properties of the agent, with what at first appeared to be unreliable results as a consequence of uncertain symptom expression. In some, but not in all instances, mild symptoms were secured on the first transfer from the original inoculated bean to bean, but not on the second transfer from the bean.

It was not until the results of certain property studies were completed that it became clear that a virus was not concerned with the

symptoms in question, e. g., the Tangier pea extract was found to withstand autoclaving at 115° C. for 30 minutes or longer without any reduction of its ability to reproduce the typical effect on the bean plant. The substance was not harmed by long aging in extract,

FIGURE 1.— *A*, Allergic type of reaction on bean plants inoculated with extract from healthy Tangier peas, showing typical mild stunting and chlorosis on first trifoliate leaves. Note absence of injury to inoculated primary leaves. *B*, Uninoculated control plants.
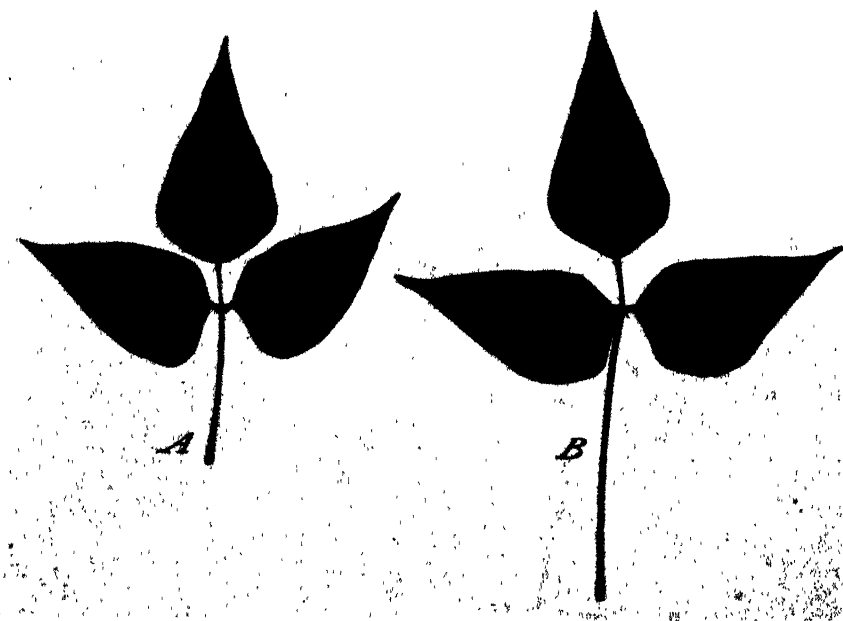
FIGURE 2.—*A*, Trifoliate leaf of bean showing mild stunting, general chlorosis, and some vein-clearing in response to the inoculating of the primary leaves with extract of the Tangier pea. *B*, Leaf from uninoculated control plant.

by freezing, or by drying for several months. The extract, however, rapidly lost its activity on dilution with water beyond 1 to 25. The active substance is filterable and capable of dialyzing through osmotic membranes. It is entirely extractable with water from the tissues

FIGURE 3.— *A, Lathyrus pusillus; B, L. tingitanus* (Tangier pea). Inoculation to bean plants with extract from the former species yielded the bean leaf wilt virus, and inoculation with the latter produced an allergic reaction in the plants.

of the Tangier pea, but nothing is known of its solubility in other substances or its reaction to chemicals, except that it appears to be denatured by cow's milk. The product does not appear to be present

in the seed, but it soon develops in the germinating seed. Symptoms similar to those produced on the Refugee bean were secured on other bean varieties, although only doubtful signs were observed on the varieties Robust and Great Northern. Velvetbeans, soybeans, and cowpeas were affected, but some other legume species, including *Phaseolus lathyroides* L., were not. *Lathyrus pusillus* L. appears to contain the same agent as the Tangier pea, but in lesser amounts; it does not appear to be present in *Lathyrus hirsutus* L.

The behavior of the extract from the Tangier pea suggests that it is a toxic substance, but it should be explained that no appreciable effect or symptom is observable on the primary leaves to which it is applied, the symptoms developing only on secondary leaves formed later. The symptoms on the bean are, therefore, systemic rather than local in character. The active substance is not toxic to viruses, bacteria, or fungi, according to preliminary trials. Since Tangier pea hay is occasionally grown for fodder, it is presumably not toxic to animals.

Although no further investigation of the Tangier pea extract was made, the writer is inclined to interpret the results as an allergic response, despite the fact that such a reaction has apparently not been previously described on plants. Hassis (4) has also noted chlorotic symptoms on tulip plants inoculated with healthy narcissus juice, even when heated to 100° C. for 20 minutes before inoculation. This symptom is therefore believed to be due to some cause other than a virus, although no further interpretation of the nature of the response is suggested. According to Rowe (9), Doerr's generally accepted definition of allergy includes "all abnormal and specific reactions of a body to foreign ordinarily innocuous substances." The action of the Tangier pea extract on the bean plant, everything considered, approaches such a reaction more nearly than that of any other biological response with which the writer is acquainted.

Although the experiments with the Tangier pea were disappointing and delayed progress on the original problem, they nevertheless encouraged further investigations on the viroplasm theory. This allergic type of behavior, peculiarly, appears to be an intermediary stage between a demonstrable and a hypothetical phenomenon, since it shows that a metabolic substance from one plant can be systemically introduced into another plant and thereby set up a series of reactions which suggest some small degree of transmissibility.

THE BEAN LEAF WILT VIRUS

During the course of the inoculations to the bean with extract from other apparently healthy legume species, two viruses were found which appear to be new and distinct from other known legume viruses. Two additional viruses were secured which appeared to be mild strains of ordinary bean mosaic; consequently their origin in the experiments was more uncertain, and they were not studied in further detail. It is logical to believe that all these viruses preexisted, and that they were transmitted through the seed of the host plants, which were also acting as symptomless carriers. No other claim is made at this time in the absence of further positive proof, but the viruses themselves are of interest and will be briefly described.

One of the viruses, to which the writer has applied the common name "bean leaf wilt virus," was obtained from *Lathyrus pusillus*

(fig. 3). This species showed no signs of disease at any time, but the plants were small for favorable observation. The virus was secured from only about 25 percent of the plants tested. The symptoms on Refugee bean are largely limited to the primary inoculated leaves. which usually wilt and dry up without developing preliminary necrotic lesions (fig. 4), although diffuse types of lesions may follow the use of low concentrations of the virus in the inoculum (fig. 5). The symptoms usually require 5 to 7 days to become distinct on the primary leaves, and although the virus moves into the secondary or trifoliate leaves, it rarely causes symptoms on these parts, except a slight stunting such as may be due to a mild allergic reaction of the type mentioned above. On plants nearing maturity, a general re-
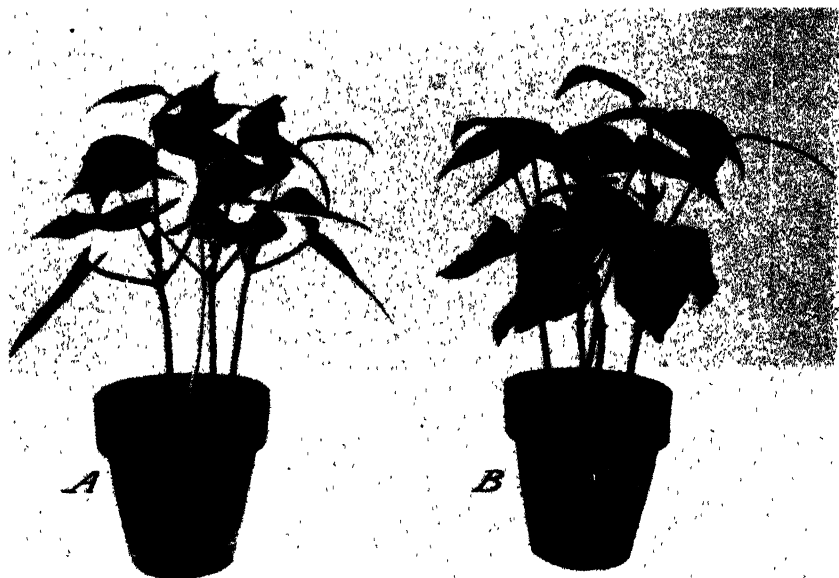
FIGURE 4.—The bean leaf wilt virus: *A*, water inoculated control bean plants; *B*, bean plants inoculated on the primary leaves with the bean wilt virus, resulting in gradual wilting and necrosis of the inoculated leaves but with little effect on the new trifoliate leaves.

tardation of growth vigor is apparent. That the virus is present in the secondary leaves may be shown by transfer.

The properties of the bean leaf wilt virus were determined to be approximately as follows: Thermal death point, 48°–50° C. at 10-minute exposures; tolerance to aging in vitro over 24 hours, in dried leaves over 30 days; tolerance to dilution over 1-1,000.

Preliminary trials indicated that the virus was transmissible by the aphid *Myzus persicae* (Sulz.), small local lesions being formed at the point of feeding by the aphids.

THE BEAN YELLOW NECROSIS VIRUS

The bean yellow necrosis virus was obtained from inoculations to the Refugee bean with young, apparently healthy *Sesbania macrocarpa* Muhl. in two separate trials, but was not secured in several other

trials.   The common name "bean yellow necrosis" virus is descriptive of the symptoms on the bean, because both yellowing and necrosis are very marked.   The secondary leaves formed soon after the inoculation of the primary leaves are often devoid of green color (fig. 6)
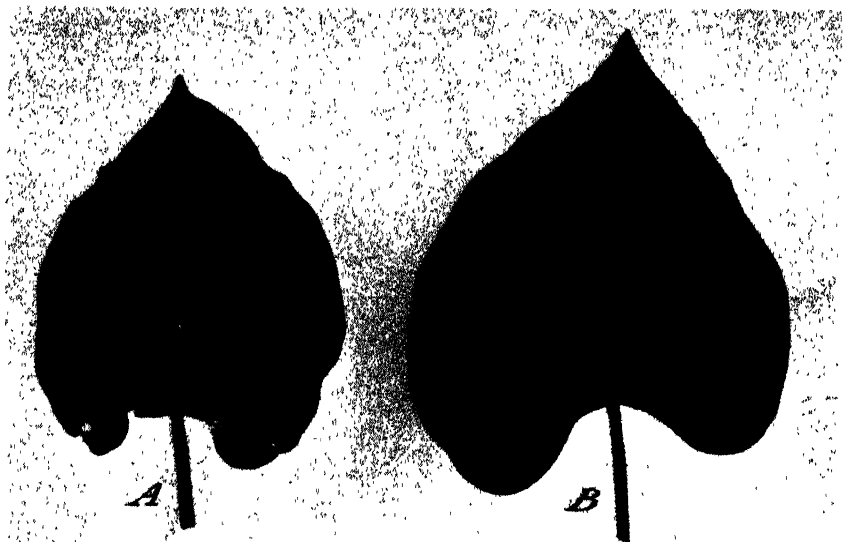
FIGURE 5.—The bean leaf wilt virus: *A*, A primary leaf of the bean inoculated with a low concentration of the bean wilt virus, resulting in a diffuse type of local lesions; *B*, primary leaf from control plant inoculated with water only.

FIGURE 6.—The bean yellow necrosis virus: *A*, Leaf from uninoculated control plant; *B*, almost completely chlorotic leaf from inoculated bean plant; *C*, milder symptoms of chlorosis and vein clearing on right side of trifoliate leaf.

and fail to grow. If young at the time of inoculation, the entire plant may become completely necrotic (fig. 7). Older plants or more resistant varieties may, however, show comparatively mild symptoms, although local necrotic spots on the inoculated primary leaves and mild chlorosis of the secondary leaves are still characteristic. The Refugee bean appears to be relatively susceptible and the Great Northern bean relatively resistant to this virus. Other legume species are susceptible, including the soybean (*Soja max* L.) Piper and the garden pea (*Pisum sativum* L.) (variety Marvel), although the virus appears to be confined to the inoculated leaves of the latter species.

FIGURE 7.—The bean yellow necrosis virus: *A*, Uninoculated control plants, *B*, bean plants inoculated in the primary leaf stage with yellow necrosis virus.

The thermal death point of the bean yellow necrosis virus was found to be 49° C. at a 10-minute exposure. It survives aging in vitro for 48 hours and possibly longer, and in dried leaves for 12 days or longer. It withstands a dilution of 1 to 100,000 and possibly as high as 1 to 500,000. The virus was removed from filtrates by both Mandler and Seitz filters. Cow's milk, which readily inactivates the ordinary tobacco virus, strangely enough does not prevent infection of bean with the bean yellow necrosis virus.

The virus is not transmitted by the aphid *Myzus persicae*, according to results of the present limited trials.

DISCUSSION

The virus diseases of the legumes have been the subject of considerable study in recent years, and the known information on the bean and pea viruses in particular has been summarized by Pierce (*8*) and Zaumeyer (*10*). It has not been possible to make a detailed

laboratory comparison of the viruses described in this paper with the several previously described legume viruses. Only bean virus 1 (ordinary bean mosaic virus) has been compared throughout the tests, largely as a control on transmission and property studies. Judging from the literature, however, the bean leaf wilt virus and the bean yellow necrosis virus appear to have little or no similarity in either symptoms or properties with the earlier described viruses on either bean or pea. The thermal death point of both new viruses is below 50° C., whereas those of the other legume viruses range from 56° to 70°. While the other properties are less consistently different, they are sufficiently so in specific instances to justify the belief that the two viruses isolated from apparently healthy plants of *Lathyrus pusillus* and *Sesbania macrocarpa* have not been previously described.

It is not surprising that such viruses should be found to exist in view of what is known about symptomless carriers of viruses. Nor is it improbable that these same viruses may be responsible for distinctive symptoms on other legume species in nature, occurring only incidentally on *Lathyrus* and *Sesbania*.

A logical proof of the relation of these viruses to the hosts from which they were secured would naturally consist of attempts to transmit the viruses back to the respective hosts from which they were obtained. Unfortunately, these efforts were not carried to a satisfactory conclusion, due in part to the uncertainty that healthy plants were available for inoculation and the unfavorableness of *L. pusillus* as a test plant, and in part to the accidental loss of the yellow necrosis virus during the summer period when the experimental work was in abeyance. The limited trials which were made indicated that the respective hosts could be infected, but that no symptoms were obtainable.

Even though the preexistence of the new viruses described is accepted, and the large number of negative results from the cross-inoculations made between different legume species (119 out of 122 species) are taken into account, a negative conclusion on the viroplasm hypothesis is not yet justified. In the earlier plans for testing the viroplasm theory, it was believed that 100 percent compatibility from any one species would be required to yield positive proof, and that if success were not secured in the first or second attempt at transfer, no further trials were necessary. It now seems quite possible that some unknown circumstance may permit a successful transfer of normal plant protoplasm from one species to another in only rare instances and still be sufficient to account for the number of known viruses. Furthermore, positive results should perhaps not be expected following artificial transfers of plant extract, such as were made in the present experiments. It is more likely that such natural means of inoculation as insect transmission may be first required before the agent concerned becomes adapted to other forms of transmission. Investigation by such methods admittedly would be laborious.

The challenge of the origin of viruses is, however, a goal worthy of unusual efforts. Many keen observers have been led to the belief that new viruses (as contrasted with virus strains which arise as variants) may originate de novo. This is quite as understandable as the assumption that certain of these extremely delicate forms of life have survived eons of time on the evolutionary basis such as applies to the origin of other forms of life. On the assumption of a de novo

origin, the relationship of foreign protoplasm (viroplasm) seems more probable than any other precursor yet described when the ability to reproduce is to be considered.

The reproduction of some protoplasmic constituents (or living protein molecules) of one species in another is not more inconceivable to science than were certain phenomena occurring in symbiosis, parasitism, allergy, graft hybrids, or sexual hybridization before they were first recognized. To be sure, additional difficulties arise in attempting to explain the relation of the viroplasm to insect vectors, but this is no more curious than the remarkable effect of insect incitants on plant tissues, as in plant galls.

The viroplasm hypothesis does not differ greatly from certain other hypotheses which have been promulgated since; e. g., that of Baur (*1*), which assumes some sort of incitant or precursor or abnormal gene (*3*) originating within the host in which the disease first occurred. The viroplasm theory suggests only that this incitant comes originally from a plant closely related to or quite foreign to the host, with the production of abnormal protein molecules, modified cytoplasmic inclusions (*5*), and usually but not always macroscopic symptoms of disease which are transmissible by virtue of the original compatability of certain species in this particular direction.

SUMMARY

Inoculations with extracts from healthy plants to other healthy plants were performed to test the hypothesis that some part of the protoplasm of one species properly introduced into the living cells of another species might find conditions compatible for growth and bring about the abnormality known as a virus disease. This possibility is referred to as the "viroplasm hypothesis."

Extracts from 122 species of legumes representing 50 genera were transferred by the wiping method of inoculation to bean (*Phaseolus vulgaris*) plants, and in some instances to other species. No symptoms were secured from 119 of the species tested. One species, *Lathyrus tingitanus*, regularly yielded symptoms, but these are interpreted as an allergic response. *Lathyrus pusillus* frequently yielded a virus which is new and which is described under the common name of "bean leaf wilt virus." *Sesbania macrocarpa* on two occasions yielded a new virus, described under the common name of "bean yellow necrosis" virus. From present knowledge of symptomless carriers, it seems most likely that the two new viruses preexisted in the hosts and that they did not originate as a result of the transfer of healthy protoplasm to another species.

Although these limited preliminary trials are not regarded as furnishing proof of the validity of the viroplasm hypothesis, they suggest other means of testing the hypothesis and may serve to aid in the interpretation of the experimental results obtained.

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EFFECT OF TRACE ELEMENTS ON GROWTH OF ASPERGILLUS NIGER WITH AMINO ACIDS [1]

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INTRODUCTION

A necessary preliminary to investigation of the effect of deficiencies in trace elements on the growth of *Aspergillus niger* Van Tiegh. with the amino acids is a study of the nutritive properties of these acids. Information in this respect is quite limited and difficult of interpretation because of differences in experimental conditions. Extensive tests, therefore, were made relative to the effects of substituting amino acid nitrogen for inorganic nitrogen prior to the study of trace-element omission. The evidence concerning participation of trace elements in enzymatic deaminations rendered it probable that their omission from the nutrient solution containing amino acids would lead · to specially marked decreases in yield. These specific growth effects would be expected to vary with the amino acid and be in agreement with the results obtained on enzyme activation. Modifications in nutrition should, on this basis, be of help also in identifying unsuspected trace-element requirements when specific needs are emphasized in this manner. This procedure has already proved successful with molybdenum (*22*) [3] and scandium (*23*).

The investigations reported here were planned with a view to obtaining information in regard to the process by which amino acids are synthesized in the plant.

Little has been published concerning the mechanism of amino acid synthesis. Kossowicz (*13*) has suggested that nitrogen enters organic combination as nitrate. Baly, Heilbron, and Stern (*2*) consider this process to be one of condensation with formaldehyde to form hydroxamic acid. Lemoigne, Monguillon, and Desveaux (*14*) have attempted to demonstrate that it is hydroxylamine that first enters organic combination. This theory has been strengthened recently by the work of Virtanen and Laine (*33*), who consider that nitrogen fixation by *Rhizobium* occurs through formation of hydroxylamine and its combination with oxalacetic acid to form the oxime of *l*-aspartic acid. The writer (*24*) has pointed out some objections to the hydroxylamine and nitrite theories that would seem to favor the ammonia theory of amino acid synthesis. The ammonia theory has been modified by Björkstén (*5*) to include the intermediate formation of an acid amide, whose condensation with acrylic acid and its homologues results in amino acid formation subsequently. Still another suggestion is that

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the amino acids are formed through decomposition of a hexose-ammonia complex.

It would seem quite certain that nitrogen metabolism in the plant is by no means static, but that synthesis of amino acids is accompanied by a simultaneous break-down of proteins already formed (7). Whether this occurs through complete decomposition of specific proteins or only of specific components of these proteins is as yet not clear. This process would seem to take place during growth as well as where maintenance only is concerned. The isotope technique employed by Vickery and associates (32) would seem well adapted to the solution of this and similar problems.

Information on the amino acids synthesized by *Aspergillus niger* is incomplete, since few investigators have concerned themselves with this subject. Abderhalden and Rona (1) were able to isolate and identify *d*-alanine, *l*-aspartic acid, *d*-glutamic acid, glycine, and *l*-leucine. Vorbrodt (34) reported the presence of tyrosine and probably phenylalanine and proline. Skinner (21) obtained a test for tryptophane. It seems likely that many, if not all, of the other known amino acids will also be found on further investigation. Takata (29), for example, reports cystine, arginine, histidine, and lysine to be present in *A. oryzae*, a form related to *A. niger*.

Previous investigations with *Aspergillus* have disclosed that inorganic nitrogen is brought into organic combination by this fungus as ammonia (24) and inorganic sulfur as sulfinic (sulfoxylic) acid (26). The organic compounds initially formed are presumably amino acids, since it has long been known that certain of these may serve as sole sources of nitrogen supply. In addition the writer (26) has shown that cysteine, cystine, or methionine may be used to fully replace sulfate sulfur. Whether all amino acids are capable of serving as sole sources of nitrogen for *Aspergillus* is unknown.

Aspergillus is able to produce its amino acids from ammonium salts and glucose indefinitely, though it is able also to use a wide variety of organic nitrogen compounds (8, 30, 31). Nutrition studies on amino acids with *Aspergillus* are in effect tests to learn the extent to which reutilization of these anabolites may occur. According to Butkewitsch (6) and Tamiya and Usami (30), utilization of amino acid nitrogen is preceded by deamination. Gorter (10) was unable, however, to detect the presence of specific deaminases and concluded deamination takes place under the influence of cell respiratory enzymes.

The tentative working hypothesis was adopted that such amino acids as are directly synthesized from hexose and ammonia would be most readily assimilable as general sources of nitrogen and of carbon. These first-formed and therefore "primary amino acids" are considered the probable precursors of the other and therefore "derived amino acids." Since the latter, according to this view, would be produced through a series of more or less complicated transformations, their assimilation would probably be more complex and therefore slower. That is to say, those amino acids closest in composition to the forms first synthesized by the fungus should prove most suitable as general sources of supply. The truth or falsity of this concept of amino acid synthesis will be considered in connection with the experimental data as it is presented.

The primary amino acids would be those formed through amination of carbon chains resulting from oxidative degradation of the sugar molecule. The extent, therefore, to which the carbon structure of the amino acids may be reutilized should serve to give some indication concerning the process of sugar fragmentation. This would be particularly the case in the event that a single amino acid is the precursor to all others (*33*).

Most investigators will probably agree that those amino acids containing the phenyl or other cyclic groups or branched carbon chains do not originate as the result of a primary synthesis from hexose. It may be anticipated, therefore, that amino acids containing these components should prove to be poor sources of nitrogen and carbon and fall into the group of derived acids.

It is certain that not all organisms can be satisfied in their nitrogen requirements by ammonium salts. In a summary by Rose (*19*) it is reported that rats are unable to synthesize *d*-valine, *l*-leucine, *d*-isoleucine, *d*-threonine, *d*-lysine, *l*-tryptophane, *l*-histidine, *l*-phenylalanine, and *l*-methionine. These, together with *d*-arginine, are considered by him to be indispensable for maximum normal growth of the rat. Either isomer of tryptophane, histidine, phenylalanine, and methionine is satisfactory for growth. Inability of the rat to synthesize these amino acids would seem due in some instances to an incapacity for introducing the amino group or forming the appropriate hydroxy or keto carbon structure (*20, p. 992*). So far it has been possible to replace leucine, isoleucine, tryptophane, histidine, and phenylalanine individually with appropriate carbon compounds without interfering with growth. Nevertheless, lysine could not be replaced in this manner.

Mueller and Kapnick (*16*) have compared their results on amino acid requirements of diphtheria bacteria with those of Fildes and Richardson (*9*) on the requirements of *Clostridium sporogenes*. Diphtheria strain "HY" required *valine*,[4] *methionine, tryptophane, histidine,* glycine, cystine, and glutamic acid. Diphtheria strain "P-W No. 8" required *valine, leucine, methionine*, cystine, and glutamic acid. *C. sporogenes* required *leucine*,[4] *tryptophane, phenylalanine, histidine, methionine, valine*, tyrosine, *arginine*, and cystine. To quote Fildes and Richardson (*9*), "It is interesting to note that these amino acids are almost the same as those involved in animal nutrition."

These data on amino acid nutrition of bacteria are of special interest, since these organisms have been found susceptible of training and adaptation to utilization of ammonium nitrogen, and vice versa. Knight (*12*) has included an extensive review of this subject in his book.

According to Butkewitsch (*6*) the first step in utilization of amino acid nitrogen by *Aspergillus* takes place through deamination. The types of deamination known to occur with micro-organisms have been summarized by Stephenson (*28*) in her book on bacterial metabolism. Experiments by Tamiya and Usami (*30*) with *A. oryzae* led to similar results on deamination with (*isoleucine*),[5] (*arginine*), (serine, *leucine*, glutamic acid, *tryptophane*, proline), (glycine, alanine, *valine*, aspartic acid), (*phenylalanine, histidine*), and (*lysine*). Urea was formed in

[4] The compounds italicized here and elsewhere in this paper are indispensable in the nutrition of the rat.
[5] The groups in parentheses are arranged in the order of decreasing growth when used as complete sources of both carbon and nitrogen.

addition to ammonia with arginine, in a manner presumably analogous to that in which it is produced in the animal.

The order specified for efficiency in amino acid utilization by Tamiya and Usami is based on supply levels ranging from N/3 to N/23. Moreover, no provision was made to insure an adequate supply of trace elements. It can be anticipated, therefore, that the order of relative utility determined for *Aspergillus oryzae* will undergo some revision in the future.

Assimilation of amino acid nitrogen by yeast was found by Nielsen (*17*) to be best with glycine, alanine, tyrosine, *leucine, isoleucine,* aspartic acid, glutamic acid, and proline. The carbon source was sucrose, supplemented by traces of beer wort. Among the poorest sources of nitrogen supply were *histidine, lysine,* and hydroxyproline. Another study by Nielsen (*18*), this time with *Rhizobium,* gave best growth at a nitrogen level of 0.002 molar with glycine, alanine, cysteine, aspartic acid, glutamic acid, *arginine, histidine,* ornithine, and *lysine.* Racemic amino acids were employed in double concentration. Sucrose was used to supply carbon, and was supplemented with biotin.

Beaumont and associates (*3*) were unable to obtain good growth of Havana tobacco when grown under aseptic conditions in water culture with alanine, glycine, *histidine, leucine, phenylalanine,* tyrosine, aspartic acid, glutamic acid, *arginine, lysine,* and proline. Asparagine and cystine were usable but inferior to inorganic nitrogen or urea.

The amino acids essential for growth of the heterofermentative lactic acid bacteria (*36*) are *valine, methionine, threonine, phenylalanine, arginine, lysine, leucine* or *isoleucine,* tyrosine, alanine, cystine, glutamic acid, serine, and aspartic acid. *Tryptophane* was essential for one strain.

EXPERIMENTAL METHODS

Aspergillus niger Van Tiegh. (No. 4247)[6] was grown for 4 days at 35° C. in each instance. The nutrient solution used contained the following compounds per liter: Sucrose, 50 gm.; ammonium nitrate, 1.90 gm.; dipotassium phosphate, 0.35 gm.; and magnesium sulfate $(7H_2O)$, 0.25 gm. Iron, zinc, copper, manganese, molybdenum, and gallium were added in concentrations of 0.30, 0.30, 0.075, 0.075, 0.02, and 0.02 mg. per liter, respectively. Water used in the preparation of this solution was redistilled in quartz. The 200-cc. Erlenmeyer culture flasks were of transparent quartz as were all other laboratory ware used. Pyrex Erlenmeyer flasks were used for work not dealing with trace elements. Each culture contained 50 cc. of nutrient solution. Further details of technique will be found in a previous publication (*22*).

Trial of nitrogen utilization of amino acids was made by substituting the amino acid for ammonium nitrate in the above formula without alteration in the nitrogen content of the solution. The nitrogen level in all cases was 665 mg. per liter or 33.25 mg. per culture, which is capable of giving a yield of 1,100–1,300 mg. per culture of 50 cc. under the conditions used.

Estimation of carbon utilization could not be made at the carbon level (50 gm. sucrose per liter) necessary for maximum yield, in view of the expense for these quantities of amino acids. Therefore the amino acids were used at a level of 10 gm. per liter. Results on

carbon assimilation are tabulated in terms of grams of yield per gram of carbon available in the substrate. The carbon-utilization factor for sucrose is 1.17 gm. of yield per gram of carbon. These values are growth rates and not absolute values on efficiency in conversion. The latter would necessarily be based on carbon actually assimilated and not on carbon supplied.

RESULTS

UTILIZATION OF AMINO ACID NITROGEN

Complete replacement of inorganic nitrogen by amino acid nitrogen (table 1) took place with alanine, arginine, aspartic acid, glutamic acid, glycine, hydroxyproline, and proline. These, together with ornithine (table 2), are by definition, therefore, the first-formed or primary amino acids for *Aspergillus*. Imino nitrogen of arginine, hydroxyproline, and proline was employed as effectively as amino nitrogen in the other acids. Moderate utilization of nitrogen occurred with serine, threonine, and tryptophane, corresponding roughly to 50-percent assimilation (27). Growth was still poorer with isoleucine, leucine, methionine, phenylalanine, tyrosine, and valine; and very poor with the remainder of the natural amino acids. These results are believed to bear no relation to isomerism, nor to form of nitrogen union. Repetition of the trials, with those amino acids (particularly serine and threonine) employed as racemic mixtures, would be required to prove this opinion. Except for hydroxyproline and proline, which contain the readily opened pyrrolidene ring, none of the amino acids containing a cyclic group or branched carbon chain fully replaced inorganic nitrogen.

TABLE 1. *Growth attained by Aspergillus niger with amino acid nitrogen (665 mg. per liter) after 4 days at 35° C.*

Amino acid	Yield	Sporulation [1]	Acidity		Starch in mycelium [2]
			Initial	At harvest	
	Mg.		*pH*	*pH*	
dl-α-Alanine	1,115.8	10	6.79	2.06	0
d-Arginine hydrochloride	1,252.4	10	6.81	1.83	2
l Aspartic acid	1,325.1	10	2.88	2.14	0
l-Cystine	71.0	1		2.25	0
d-Glutamic acid	1,292.8	10	3.18	2.04	1
Glycine	1,234.8	10	6.35	1.99	0
l-Histidine dihydrochloride	120.9	4	2.08	1.93	0
l-Hydroxyproline	1,147.4	10		2.17	1
l-Iodogorgoic acid	.0	0			
dl-Isoleucine	246.2	4		2.88	0
l-Leucine	373.6	6	6.49	2.87	0
d-Lysine dihydrochloride	16.2	0	1.99	1.97	1
dl-Methionine	437.0	7	7.44	2.85	0
dl-Norleucine	113.8	1		2.96	0
dl-Phenylalanine	370.8	4		2.78	2
l-Proline	1,110.7	10		1.93	2
dl-Serine	608.6	7		2.60	0
dl-Threonine	647.4	6		2.30	0
l-Tryptophane	636.3	6		2.61	1
l-Tyrosine	196.1	10		2.85	0
dl-Valine	291.8	1		2.78	0
Cysteine hydrochloride	12.2	0	6.28	5.96	3*
β-Alanine	18.7	4		5.33	0
Lanthionine [3]	92.3	3		2.72	0
Cystine, optically inactive [3]	98.6	1		2.47	3*

[1] Sporulation is rated from 0 (sterile) to 10 (black with spores). In some instances both black and brown (bb) or brown (br) spores were present. Where no notation is made, the spores were the normal black.
[2] Quantity of starch present in the mycelial felts is based on a scale of 0 (none) to 5 (maximum). An immediate blue color with N/20 iodine is indicated by an asterisk (*). When starch was also present in the substrate it is given in parentheses on the same scale.
[3] Obtained through the kindness of Dr. D. B. Jones and Dr. M. J. Horn, of the Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture.

TABLE 2.—*Growth attained by Aspergillus niger with miscellaneous nitrogen compounds (665 mg. nitrogen per liter) after 4 days at 35° C.*

Compound	Yield	Sporulation [1]	Acidity		Starch in mycelium [2]
			Initial	At harvest	
	Mg		*pH*	*pH*	
dl-β,β-Phenylalanine	2.7	0		3.67	0
S-benzylcysteine [3]	63.5	2			0
α-Amino-*n*-butyric acid	605.2	4		3 32	0
β-Amino-*n*-butyric acid	16 2	4		3 38	0
α-Amino-isobutyric acid	.6	0		3.67	0
ε-Amino-*n*-caproic acid	8.0	0		4.88	0
Taurine	75.8	2, br	7.23	2.78	0
d-Glucosamine hydrochloride	446.5	10		1.63	2 (4)
dl-Homomethionine [4]	1.0	0	7.23	3.52	0
Cysteic acid [5]	17 6	2		1.68	0 (1)
Cysteine disulfoxide [5]	81 6	0	7.23	1.89	5 [6] (1)
β-Hydroxyethylamine [6]	.0	0	7.23		
N-Phenylglycine	6	0	7 23	5.05	0
dl-Benzoylalanine	346.2	4	7.23	2.72	0
dl-α-Aminophenylacetic acid	155.0	2	7.23	2.53	0
Sarcosine	208.5	10	7 23	2.53	0
dl-Alanine ethyl ester hydrochloride [7]	228.0	4	7.23	1 82	4 (3)
Glycine ethyl ester hydrochloride [7]	531.0	8	7 23	1 58	0
dl-α-Amino-*n*-valeric acid	80.9	2	7 23	3.00	0
δ-Amino-*n*-valeric acid hydrochloride	181 0	6	7 23	1.73	0
d-Ornithine hydrochloride	1,017.3	10	7 23	1 47	5 [*]
dl-α-Amino-α-methylbutyric acid	1.4	0	7 25	3 37	0
Creatine	1 6	0	7 23	4 35	0
Ethylamine hydrochloride	21 8	1	7 23		0
Methyleneamineacetonitrile	.0	0	7 25		0

[1] See table 1, footnote 1.
[2] See table 1, footnote 2.
[3] Obtained through the kindness of Dr. Ben H. Nicolet, of the Bureau of Dairy Industry, U. S. Department of Agriculture.
[4] Obtained through the kindness of Prof. Vincent du Vigneaud, Cornell University Medical School, New York, N. Y. Yield with 50 mg. (instead of 380 mg.) of *dl*-homomethionine per culture. At a sulfur level of 25 mg. per liter, the yield was only 8.0 mg. Therefore utilization of both nitrogen and sulfur was quite poor.
[5] Obtained through the kindness of Dr Grace Medes, of the Lankenau Hospital, Philadelphia, Pa.
[6] Growth takes place on β-hydroxyethylamine if the substrate is strongly acidified with sulfuric acid prior to sterilization. Starch formation in the mycelial felts would indicate the main product of decomposition under these conditions to be ammonia.
[7] The ethyl esters of glycine and alanine gave variable results but never greater than that equivalent to a 50-percent utilization. Variations are attributed to differences in degree of hydrolysis.

The amino acids capable of fully replacing inorganic nitrogen with *Aspergillus* are all among those classified by investigators of animal physiology as glycogenic (*20*), i. e., capable of conversion into glucose by animals. None are ketogenic (*20*), or acetone precursors. In no instance did an amino acid incapable of synthesis by the rat serve to fully replace inorganic nitrogen with *Aspergillus*. Primary amino acids, or those capable of complete reutilization by *Aspergillus*, are also those capable of synthesis by the rat. Those classified as indispensable amino acids for the rat, except possibly arginine, fall into the derived amino acid group with *A. niger*.

Attention is called to the enhanced production of starch by cysteine even when practically no growth is made. It would be of interest to learn whether cysteine plays a specific role in this process or whether its action is indirect.

None of the sulfur derivatives of the amino acids were capable of providing appreciable assimilable nitrogen with the exception of methionine (tables 1 and 2). The cause of this failure does not seem related to the state of oxidation of the contained sulfur inasmuch as the mercapto group in cysteine and the sulfonic acid group in cysteic acid were equally effective in rendering nitrogen unassimilable. The marked superiority of methionine as a source of nitrogen, compared

with cysteine (cysteine/alanine=0.011, methionine/α-amino-*n*-butyric acid=0.722), was attributed to the additional —CH₂— group separating sulfur and nitrogen. Homomethionine, which contains two additional —CH₂— groups, proved practically valueless, however, as a source of either sulfur or nitrogen (homomethionine/α-amino-*n*-valeric acid=0.110). Substitution for mercapto hydrogen did not increase nitrogen assimilability (S-benzylcysteine).

Full utilization of amino acid nitrogen in the compounds listed in table 2 occurred only with ornithine. Approximately 50-percent utilization took place with *dl*-α-amino-*n*-butyric acid and *d*-glucosamine hydrochloride. Amino nitrogen in the beta position or in caproic acid (α-NH₂, ϵ-NH₂, α, ϵ-NH₂) was unassimilable. Neither α-amino nor δ-amino nitrogen of *n*-valeric acid could be used, whereas ornithine (α, δ-NH₂) gave maximum growth. Substitutions for amino hydrogen and carboxyl hydrogen gave decreased nitrogen utilization. Reduction of an acid group (—COOH) to an alcohol group (—OH), as, for example, glycine to β-amino ethyl alcohol, prevented nitrogen assimilation. These results are discussed more fully in the section dealing with enzyme action.

The relative unavailability of nitrogen in the derived amino acids was attributed in the discussion of table 1 to the difficulty with which they are deaminated. The possibility exists, however, that toxicity might be a factor in these responses. In order to eliminate this factor as completely as possible, the experiments were repeated with the derived amino acids in the presence of ammonium nitrate. Since maximum yields are given by ammonium nitrate in concentrations much higher than those employed in the standard solution, it was considered that growth at double the nitrogen level should also be maximum. Table 3 gives the data for yields at a nitrogen level of 1,330 mg. per liter, of which one-half was in the form of ammonium nitrate and the other half an amino acid.

Table 3.- *Growth attained by Aspergillus niger after 4 days at 35° C. when supplied with 665 mg. of nitrogen as ammonium nitrate and an equal quantity as derived or synthetic amino acid*

Compound	Yield per 2.5 gm. sucrose	Sporulation [1]	Starch in mycelium [2]
	Mg.		
Ammonium nitrate	1,240. 0	2	5*(5)
β-Alanine	1,157. 0	10	0
dl-α-Amino-*n*-butyric acid	1,203 6	10	3
β-Amino-*n*-butyric acid	735. 6	10	0
dl-α-Amino-isobutyric acid	785 2	6	0
ε-Amino-*n*-caproic acid	705 8	10	0
Cysteine hydrochloride	257 6	0	5*
l-Cystine	1 151 8	10	1
l Histidine dihydrochloride	1,169 9	2	3(E)
dl-Isoleucine	892 2	4	0
l-Leucine	827. 2	10	1
d-Lysine dihydrochloride	1,263. 8	4	2(2)
dl-Methionine	919. 8	9	0
dl-Norleucine	1,004. 8	10	0
dl-Phenylalanine	1,204 6	9	0
dl-β, β-Phenylalanine	1,047 1	10	1
dl-Serine	1,154. 3	6	0
dl-Threonine	1,274. 2	10	0
l-Tryptophane	1,195. 7	7	0
l-Tyrosine	1,247. 7	8	1
dl-Valine	1,104. 3	8	0

¹ See table 1, footnote 1.
² See table 1, footnote 2. A red coloration with iodine solution is indicated by "E," abbreviation for erythrodextrin.

Under these conditions the only amino acids of natural occurrence which did not give maximum yields were cysteine, isoleucine, leucine, and possibly methionine. However, these amino acids gave greater yields in the presence of ammonium nitrate than when used alone. The greater number of derived amino acids were nontoxic at the nitrogen levels employed. The toxicity exhibited by the above-mentioned amino acids is due perhaps to enzyme inactivation through permanent combination with aminases required for introduction of nitrogen into other amino acids. Cysteine had an especially marked action in preventing synthesis of amino acids from ammonia.

The effect of cysteine on the utilization of amino acids is shown in table 4. Its presence led to a diminution in yield on a milligram basis, particularly with the primary amino acids alanine, arginine, aspartic acid, glutamic acid, glycine, hydroxyproline, and proline. This result is in conformity with but no proof of the assumption that cysteine combines with the enzyme responsible for both the liberation of amino nitrogen supplied and the subsequent introduction of the ammonia produced to form new amino acids.

TABLE 4.—*Sporulation and yield of Aspergillus niger after 4 days at 35° C. when grown in the presence of 665 mg. of nitrogen as cysteine hydrochloride or of glycerol as carbon source*

Amino acid added in quantity equivalent to 665 mg. of nitrogen per liter	Cysteine hydrochloride 7.48 gm. per liter [1]		Glycerol as carbon source	
	Sporulation [2]	Yield	Sporulation [1]	Yield
		Mg.		*Mg*
dl-Alanine	0	431 1	8	409 8
d-Arginine hydrochloride	0	113. 7	8	119 9
l-Aspartic acid	0	404 4	0	259 3
l-Cystine	0	16 5	8	9
d-Glutamic acid	0	97 8	8	430. 5
Glycine	0	265 9	1	8. 7
l-Histidine dihydrochloride	0	158 4	8	116. 1
l-Hydroxyproline	0	31 1	4	14 2
dl-Isoleucine	0	45 7	0	2 1
l-Leucine	0	86 1	6	334 7
d-Lysine dihydrochloride	0	89 4	1	23 9
dl-Methionine	0	34 7	4	27 6
dl-Norleucine	0	79 6	1	5 4
dl-Phenylalanine	0	77 5	0	8 3
l-Proline	0	352 9	10	925 5
dl-Serine	0	99 5	1	9 7
dl-Threonine	0	52 8	1	2. 1
l-Tryptophane	0	28. 7	1	5 4
l-Tyrosine	0	19. 6	2	22. 8
dl-Valine	0	202. 0	0	6 8
Equal quantities of nitrogen as [3]				
l-Cystine+glycine	10	1, 128 9		
l-Cystine+*dl*-alanine	10	1, 101 6		
l-Cystine+*d*-glutamic acid	10	1, 301 6		
d-Lysine+glycine	10	1, 315. 9		
d-Lysine+*dl*-alanine	10	1, 270. 0		
d-Lysine+*d*-glutamic acid	10	1, 567. 1		

[1] All cultures containing 50 percent cysteine nitrogen gave starch tests equivalent to 5* (5)
[2] See table 1, footnote 1.
[3] These data indicate *l*-cystine inhibits growth only slightly whereas *d*-lysine does so not at all.

Assimilation of amino acid nitrogen when glycerol was substituted for sucrose is of interest from several points of view. Growth of *Aspergillus* on glycerol is very poor (10–400 mg.) with inorganic nitrogen. This carbon source seems most suitable for studying the effect of scandium (*23*) on yields, though inability to obtain maximum

yields with glycerol has proved a serious hindrance. The data would indicate that approximately maximum growth may be obtained on glycerol when nitrogen is supplied as proline.

Yields obtained in the presence of glycerol differ from those similarly obtained with amino acid nitrogen when sucrose is used. Growth was greatest with proline, glutamic acid, alanine, leucine, and aspartic acid, in the order named. Arginine, glycine, and hydroxyproline gave much poorer results than with sucrose, while with leucine and histidine the yields were the same. The reason for these responses is ascribed to the effect of the carbon residues of the amino acids, and the effects of carbon supplementation or admixtures in carbon supply (*23*). Greater weight must therefore be placed on the results of amino acid addition with a good source of carbon (sucrose) than with a poor one (glycerol).

Slight decreases in utilization of glycine, alanine, and glutamic acid are also brought about by *l*-cystine, but not by *d*-lysine. Assimilability of other amino acids on addition of cystine was not investigated.

AMINO ACID STRUCTURE AND ENZYMATIC DEAMINATION

A compilation of the data obtained with glycine and alanine, some of their derivatives, and a few compounds of closely similar structure will be found in table 5. Care has been exercised to omit from each series any closely related amino acids of natural origin possibly subject to the influence of a different enzyme. Exceptions to this rule are safe only if the substances are completely unassimilable. Comparisons could of course be made on a broader basis, if it were definitely known that glycine and alanine reacted with the same enzyme.

TABLE 5.— *Comparison of variations in assimilability of nitrogen by Aspergillus niger obtained through alteration of the molecular structure of glycine and alanine*

Compound	Formula	Yield	Sporulation [1]
		Mg.	
Glycine	$CH_2(NH_2)CO\,OH$	1,234 8	10
Glycine ethyl ester hydrochloride	$CH_2(NH_2.HCl)CO\,OC_2H_5$	531 0	8
Sarcosine	$CH_2(NH.CH_3)COOH$	208 3	0
dl-α-aminophenylacetic acid	$C_6H_5CH(NH_2)COOH$	155. 0	2
N-Phenylglycine	$CH_2(NH.C_6H_5)COOH$	.6	0
β-Aminoethyl alcohol	$CH_2(NH_2)CH_2OH$	.0	0
α-Amino-isobutyric acid	$(CH_3)_2C(NH_2)COOH$	.6	0
Methyleneaminoacetonitrile	$CH_2(N\cdot CH_2)CN$	.0	0
dl-Alanine	$CH_3CH(NH_2)COOH$	1,115. 8	10
β-Alanine	$CH_2(NH_2)CH_2COOH$	18. 7	4
dl-Alanine ethyl ester hydrochloride	$CH_3CH(NH_2.HCl)COOC_2H_5$	228. 0	4
dl-Benzoylalanine	$CH_3CH(NH.COC_6H_5)COOH$	346 2	4
dl-β-Amino-β-phenylalanine	$C_6H_5CH(NH_2)CH_2COOH$	2 7	0
Cysteine hydrochloride	$HS.CH_2CH(NH_2.HCl)COOH$	12. 2	0
S-Benzylcysteine	$C_6H_5CH_2SCH(NH_2)COOH$	63. 5	2
Cysteic acid	$HO_3SCH_2CH(NH_2)COOH$	17. 6	2
Taurine	$HO_3SCH_2CH_2.NH_2$	75 8	2, br
(*dl*-Serine)	$HOCH_2CH(NH_2)COOH$	608. 6	7
(*dl*-α-Amino-*n*-butyric acid)	$CH_3CH_2CH(NH_2)COOH$	605 2	4
(α-Amino-isobutyric acid)	$(CH_3)_2C(NH_2)COOH$	.6	0

[1] See table 1, footnote 1.

Glycine, the simplest of the amino acids, contains three significant groups: Carboxyl, α-amino, and α-hydrogen. Examination of the compounds listed will disclose that esterification of glycine or reduc-

tion of carboxyl inhibits utilization. Complete replacement of α-hydrogen or even partial substitution of amino hydrogen leads to a similar result.

The alanine molecule contains each of the characteristic groupings of glycine, and in addition contains β-hydrogen. It will be seen that responses to modifications in alanine parallel those with glycine. A shift of the amino group from the α to the β carbon atom also destroys assimilability. Most interesting of all, however, are the decreases in utilization brought about by replacements of β-hydrogen.

These responses parallel so closely those obtained in vitro in the study of enzyme action on the peptide (–NHCO–) group (4) that there can be little question but that both are fundamentally due to the same intrinsic type of enzymatic mechanism. The difference in results—deamination as compared to opening of the peptide linkage— is probably due to the different arrangement of these forces to insure strain and rearrangement at different points of the substrate molecules.

UTILIZATION OF AMINO ACID CARBON

No information is available concerning the oxidative break-down of sugar that leads to production of α-keto or hydroxy acids presumably required for direct amino acid formation. Even the number of carbon chains employed in amino acid synthesis is unknown as yet, although the data of table 1 would suggest that individual units for each of the known amino acids are unnecessary. Assuming that only the primary amino acids are directly synthesized from degradation products of carbohydrate, the number required would still be six. This would mean separate keto or hydroxy acids for formation of alanine, arginine (ornithine), aspartic acid, glutamic acid, glycine, and proline (hydroxy-proline), unless interconversions occur among these also.

If any basis exists for the assumption that only a few of the amino acids are of primary origin, these same acids should serve best as sources of carbon. Moreover, the extent to which the carbon of the sugar is replaceable by amino acid carbon should serve as a measure of the proportion of carbohydrate carbon employed for formation of amino acids. The rapid appearance in the substrate of ammonia when nitrates are used is evidence that larger quantities are formed than appear in the cellular material. This decomposition of amino acids is without doubt a necessary accompaniment of growth.

The data of table 6 reveal, in fact, that only certain of the primary amino acids are suitable to any degree as sources of carbon. These are arginine, aspartic acid, glutamic acid, ornithine, and proline. Alanine, glycine, and hydroxyproline were quite ineffective for this purpose. Moreover, the reality of the proposed classification of the amino acids into primary and derived groups on the basis of their suitability as sources of nitrogen is substantiated by their behavior as sources of carbon. The low efficiencies in carbon utilization as compared to sucrose (1.17) are interpreted to mean that several of the primary amino acids are formed directly from hexose and ·ammonia and the balance by subsequent simple interconversions from these. This point will be discussed shortly.

As previously mentioned, the formation of a single primary amino acid as precursor to all others would be indicated by its effectiveness

when substituted for sucrose. If several are so formed as precursors, their use in admixture should also lead to high replaceability of sucrose. Replaceability need not be complete, however, since a certain proportion of sucrose is employed for purposes other than formation of amino acids. No certainty exists that amino acids can serve for effective replacement of sucrose used in respiration and fermentation.

A mixture of glutamic acid, ornithine, and proline gave a yield of 0.82 gm. per gram of carbon. This was the maximum factor obtained with any amino acid mixture. Aspartic acid could be substituted for glutamic and arginine for ornithine. This value could not be increased by addition of glycine, alanine, hydroxyproline, or leucine. An interesting and important feature of these results is the fact that glutamic acid, ornithine, and proline are all α, δ-substituted valeric acid derivatives. Interconversion probably accounts for equivalence between glutamic and aspartic acids (*15*), and the formation of the other primary acids (*35*). Admixture studies therefore also confirm the hypothesis that the amino acids first formed possess maximum assimilability.

TABLE 6.-- *Sporulation and relative carbon assimilation of amino acids by Aspergillus niger after 4 days' growth at 35° C.*

[Sucrose = 1.17]

Compound	As source of carbon		As source of carbon and nitrogen	
	Sporulation [1]	Yield per gram of carbon	Sporulation	Yield per gram of carbon
		Gm		*Gm*
dl-Alanine	1	0.02	2	0.09
d-Arginine hydrochloride	0	.10	0	.02
l-Aspartic acid	4	.21	2	.23
l-Cystine	0	00	0	00
d-Glutamic acid	4	23	1	27
Glycine	0	.00	0	00
l-Histidine dihydrochloride	0	.00	0	.02
l-Hydroxyproline	1	.06	2	07
dl-Isoleucine	0	00	0	00
l-Leucine	0	.01	0	.01
d-Lysine dihydrochloride	0	.00	0	0.
dl-Methionine	0	00	0	00
dl-Norleucine	0	00	0	00
dl-β-Phenylalanine	0	.00	0	.00
l-Proline	2, br	².34	6, br	15
dl-Serine	0	.00	0	.00
dl-Threonine	0	.00	0	.00
l-Tryptophane	0	.00	0	.00
l-Tyrosine	0	00	0	00
dl-Valine	0	.00	0	.00
Creatine	0	.00		
Cysteine hydrochloride	0	.00	0	.00
α-Amino-n-butyric acid	0	.00	0	.00
α-Amino-isobutyric acid	0	.00		
ε-Amino-n-caproic acid	0	00	0	.00
dl-α-Amino-n-caprylic acid	0	.00	0	.00
d-Glucosamine hydrochloride	1	03	2	.07
Asparagine	1	.02	0	.01
dl-α-Amino-n-valeric acid	0	.00	0	.00
d-Amino-n-valeric acid	0	.00	0	.00
d-Ornithine dihydrochloride	1	.10	2	.07

[1] See table 1, footnote 1.
² Another sample gave a value of 0.22.

ASSIMILABILITY AND MOLECULAR CONFIGURATION

In view of Gorter's (*10*) evidence that specific deaminases do not enter into these reactions, it is of interest to summarize some of the biological data from the chemical viewpoint. Table 7 shows data on the effect of the length of the carbon chain on assimilability of the α-amino monocarboxylic acids by *Aspergillus niger*. Availability as nitrogen supply decreases rapidly with increased length of carbon chain. Utilization of these acids as sources of carbon is practically nil. The introduction of an amino group or oxygen at the terminal carbon atoms of these acids increases both nitrogen and carbon utilization considerably (aspartic and glutamic acids, proline, ornithine). Lysine is an exception, but α-amino adipic acid may prove slightly more available.

TABLE 7.—*Effect of length of carbon chain on assimilability of α-amino monocarboxylic acids by Aspergillus niger*

Amino acid	Carbon chain	Yield as nitrogen source	Yield per gram carbon
		Milligrams	*Gram*
Glycine	2	1,234.8	0.00
Alanine	3	1,115.8	.02
α-Amino-*n*-butyric acid	4	605.2	00
α-Amino-*n*-valeric acid	5	80.9	.00
Norleucine	6	113.8	.00
α-Amino-*n*-caprylic acid	7		.00

TRACE-ELEMENT DEFICIENCIES IN UNPURIFIED SOLUTIONS

The effects of the omission of biologically essential trace elements from the nutrient solution containing amino acid nitrogen are shown in table 8. The amino acids included cover the full range in response by the fungus. Omission of trace elements from the nutrient solution resulted only in minor decreases in growth. This would indicate the presence of relatively large quantities of these elements as impurities or possibly a diminished requirement. Perhaps their purification is not carried to the same degree as with reagent chemicals or it may be that these impurities are retained more tenaciously by amino acids (*11*). Responses with trace-element deficiencies, however, took place with each amino acid that was used.

TABLE 8.—*Effects of deficiencies in trace elements on growth of Aspergillus niger with amino acid nitrogen after 4 days at 35° C.*

Element omitted	Glycine					α-Alanine				
	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]
	Mg.	*Percent*	*pH*			*Mg.*	*Percent*	*pH*		
None	1,142.9	100.00	1.99	0	10	1,065.3	100.00	2.06	0	10
Fe	1,133.4	99.17	2.59	0	10	998.3	93.71	1.73	0	.10
Zn	339.7	29.72	2.63	0	10	530.5	49.80	2.58	0	10
Cu	1,234.8	108.04	2.03	0	10, br	1,115.8	104.74	1.97	0	10, br
Mn	1,092.3	95.57	1.87	0	10	984.4	92.41	2.07	0	10
Mo	1,109.5	97.08	1.97	0	10	1,089.7	102.29	2.42	0	10
Ga	1,152.8	100.87	2.00	0	10	1,086.0	101.94	2.01	0	10
Max. [3]	1,234.8					1,115.8				
C. U. [4]		49.39					44.63			
pH [5]			6.35					6.79		

See footnotes at end of table.

TABLE 8.—*Effects of deficiencies in trace elements on growth of Aspergillus niger with amino acid nitrogen after 4 days at 35° C.*—Continued

Element omitted	d-Arginine hydrochloride					l-Histidine dihydrochloride				
	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium[1]	Sporulation[2]	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium[1]	Sporulation[2]
	Mg.	Percent	pH			Mg.	Percent	pH		
None	1,236.1	100 00	1.83	2	10	89.0	100.00	1.93	0	4
Fe	364.1	29.46	2.29	1	6	63 7	71.61	1.92	0	4
Zn	432 2	34.96	2.23	1	8	80.8	90 79	2.08	0	4
Cu	1,241 6	100.44	1.83	0	10	102.9	115.6	1.85	0	4
Mn	1,179.3	95.40	1.80	1	10	33.0	37.08	1.76	3*	4
Mo	1,237.4	100.11	1 81	3	10	73 3	82.35	1.89	0	4
Ga	1,238.0	100 15	1.83	0	10	116 1	130.43	1.84	0	4
Max.[3]	1,252 4					120 9				
C. U.[4]		50.10						4 84		
pH[5]			6.81					2.08		

Element omitted	Cysteine hydrochloride					dl-Methionine				
	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium[1]	Sporulation[2]	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium[1]	Sporulation[2]
	Mg.	Percent	pH			Mg.	Percent	pH		
None	11 5	100.00	5.96	3*	0	437.0	100 00	2.85	0	7
Fe	.9	7.77	5.95	0	0	333 3	76 27	2.64	0	4
Zn	9 2	79 61	5 98	3*	0	273 1	62 49	2 62	0	9
Cu	8 2	70 87	6 02	3*	0	412 3	94.35	2 61	0	8
Mn	0	0	6 03	0	0	349 5	79 98	2 67	1	4
Mo	9 3	80 58	6 02	4*	0	431.4	98 72	2 59	0	8
Ga	9 3	80.58	6 01	3*	0	405.9	92 88	2.59	0	8
Max.[3]	12 2					437 0				
C. U.[4]		49						17.48		
pH[5]			6 28					7.44		

Element omitted	l-Aspartic acid					d-Glutamic acid				
	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium[1]	Sporulation[2]	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium[1]	Sporulation[2]
	Mg.	Percent	pH			Mg.	Percent	pH		
None	1,196 8	100.00	2.14	0	10	1,284.1	100.00	2 04	0	10
Fe	367 2	30 68	2.66	0	4	1,293.5	100 73	2.19	0	10
Zn	210 0	17 55	2 88	0	10	679 9	52 95	2 41	0	10
Cu	1,325.1	110 72	2 27	0	10, br	1,277.7	99 50	2 05	0	10
Mn	979 3	81.83	1 89	1	1	1,298 5	101 12	1 87	2	8
Mo	1,115.3	93 19	2 14	0	10	1,315 7	102.46	1.98	2	10
Ga	1,175 0	98 18	2 19	0	10	1,292 8	100.68	2.01	0	10
Max[3]	1,325.1					1,315.7				
C. U.[4]		53.00						52.63		
pH[5]			2 88					3 18		

See footnotes at end of table.

TABLE 8.—*Effects of deficiencies in trace elements on growth of Aspergillus niger with amino acid nitrogen after 4 days at 35° C.*—Continued

Element omitted	*l*-Leucine					*d*-Lysine dihydrochloride				
	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]
	Mg.	*Percent*	*pH*			*Mg.*	*Percent*	*pII*		
None	325. 4	100. 00	2. 87	0	6	14. 8	100. 00	1. 97	• 1	0
Fe	155. 1	47. 66	2. 95	0	0	14 2	96. 06	1 95	1	0
Zn	209. 2	64. 29	2. 94	0	4	13. 3	89 76	1. 94	0	0
Cu	304. 4	93. 55	2. 81	0	6	13. 9	93. 70	1. 92	1	0
Mn	373 6	114. 80	2 71	0	8	16. 2	109. 45	1 92	1	0
Mo	208. 7	64. 14	2. 99	0	2	14. 4	96 85	1 93	0	0
Ga	256. 1	78. 70	2 87	1	4	14. 7	99. 21	1. 93	1	0
Max. [3]	373. 6					16 2				
C. U. [4]		14. 94					. 65			
pH [5]			6 49					1. 99		

[1] See table 1, footnote 2.
[2] See table 1, footnote 1.
[3] Maximum individual yield.
[4] Coefficient of utilization, or yield per 100 gm. of sucrose.
[5] Initial acidity of nutrient solution.

Extent of sporulation and of shifts in acidity varied approximately with the magnitude of growth. Little formation of starch took place in the mycelial felts except after growth on cysteine. Its production occurred in this case despite the relatively low acidities following growth on cysteine. An increased acidity should lead to a further intensification in elaboration of starch (*25*).

TRACE-ELEMENT DEFICIENCIES IN PURIFIED SOLUTIONS

Additional tests to determine the effects of trace-element deficiencies on *Aspergillus niger* when grown with amino acids were carried out after purification with calcium carbonate. The results are tabulated in table 9. Improved results were obtained in many instances after omission of trace elements following nutrient solution purification. The improvements were slight, however, and poorer than those usually obtained when inorganic nitrogen is employed. These results further demonstrate the retention of trace-element impurities by the amino acids. It is possible that retention of impurities by the amino acids is to be associated with the presence of amino nitrogen and the formation of unionized complexes (*11*). Nevertheless it is clear that the trace elements needed for growth with inorganic nitrogen are also required with amino acid nitrogen.

DISCUSSION

An important phase of amino acid nutrition deals with specificity in requirements and the comparative needs of different organisms. Data in the literature would indicate that marked specificity exists with the bacteria and fungi, accompanied by marked variations between species and even strains. Animals, by contrast, do not seem to differ in amino acid requirements. It may well be that further study will reveal greater uniformity to exist among lower forms also. A contrasting variation in technique which may be partly responsible for this situation is the use of complex and effective carbon sources with animals. Lower organisms are usually grown with a single highly purified carbon source.

TABLE 9.—*Effects of deficiencies in trace elements on growth of Aspergillus niger, with amino acid nitrogen in solutions purified with calcium carbonate, after 4 days at 35° C.*

Element omitted	α-Alanine					Glycine				
	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium[1]	Sporulation[2]	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium[1]	Sporulation[2]
	Mg.	*Percent*	*pH*			*Mg.*	*Percent*	*pH*		
None	1,173.7	100.00	2.57	0	10	1,342.0	100.00	2.61	0	16
Fe	15.5	1.33	3.02	0	2	35.0	2.61	2.95	0	1
Zn	18.6	1.60	2.86	0	2	53.7	4.00	2.85	0	2
Cu	1,008.2	85.90	2.64	1	6, br	1,349.5	100.56	2.58	0	6, br
Mn	644.7	55.32	2.14	2	1	1,445.9	107.74	2.33	0	10
Mo	1,111.5	94.70	2.63	0	10	1,225.5	91.32	2.52	0	10
Ga	1,160.9	98.91	2.61	0	10	1,292.5	96.31	2.50	0	10
Max.[3]	1,219.7					1,459.8				
C. U.[4]		48.79					58.39			
pH[5]			6.85					7.83		

Element omitted	l-Aspartic acid					d-Glutamic acid				
	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium[1]	Sporulation[2]	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium[1]	Sporulation[2]
	Mg	*Percent*	*pH*			*Mg.*	*Percent*	*pH*		
None	643.4	100.00	3.02	0	10	912.4	100.00	2.78	2	10
Fe	17.5	2.72	3.64	0	0	27.5	3.01	3.62	0	1
Zn	43.6	6.78	3.13	0	2	21.3	2.33	3.35	0	1
Cu	619.4	96.27	3.01	0	10	944.5	103.51	2.58	2	10
Mn	614.5	95.51	3.01	0	10	980.5	107.46	2.26	1	10
Mo	626.6	97.39	2.99	0	10	893.2	97.90	2.76	3	10
Ga	663.8	103.17	2.92	0	10	892.0	97.76	2.71	2	10
Max.[3]	663.8					980.5				
C. U.[4]		26.55					39.22			
pH[5]			5.33					5.27		

Element omitted	l-Leucine					dl-Norleucine				
	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium[1]	Sporulation[2]	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium[1]	Sporulation[2]
	Mg.	*Percent*	*pH*			*Mg.*	*Percent*	*pH*		
None	473.4	100.00	2.85	0	1	94.5	100.00	2.82	1	0
Fe	67.3	14.27	2.90	0	0	41.9	44.38	2.65	0	0
Zn	220.8	46.28	2.80	0	6	44.7	47.34	2.67	0	0
Cu	546.6	115.40	2.81	0	1	95.3	100.83	2.77	1	0
Mn	650.9	137.74	2.87	0	1	85.0	89.94	2.79	1	0
Mo	558.6	118.90	2.83	0	1	89.3	94.56	2.78	1	0
Ga	539.7	94.80	2.87	0	1	94.6	100.12	2.80	1	0
Max.[3]	546.6					97.0				
C. U.[4]		22.34					3.88			
pH[5]			6.89					6.69		

See footnotes at end of table.

TABLE 9.—*Effects of deficiencies in trace elements on growth of Aspergillus niger, with amino acid nitrogen in solutions purified with calcium carbonate, after 4 days at 35° C.*—Continued

Element omitted	dl-Isoleucine					dl-Valine				
	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]	Yield per 2.5 gm sucrose	Relative yields	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]
	Mg.	*Percent*	*pH*			*Mg.*	*Percent*	*pH*		
None	156.7	100.00	3 11	0	4	216.4	100 00	3.00	0	2
Fe	8.1	4 54	3.20	0	1	4 7	2.17	3 41	0	2
Zn	22 3	12.45	3.00	0	2	16.5	7.61	3 06	0	0
Cu	166 1	92.87	3.09	0	4	131.8	60.92	3.09	0	1
Mn	145 8	81.52	3 11	0	4	172.4	79.67	3.10	0	2
Mo	184.3	130.48	3.02	0	4	138.9	64.20	3.10	0	2
Ga	198.6	111.02	3.04	0	4	120.0	55.43	3.09	0	2
Max.[3]	261.5					267 0				
C. U.[4]		10.46					10 68			
pH [5]			7 04					7.04		

Element omitted	dl-Threonine					l-Tryptophane				
	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]	Yield per 2.5 gm. sucrose	Relative yields	Acidity at harvest	Starch in mycelium [1]	Sporulation [2]
	Mg	*Percent*	*pH*			*Mg.*	*Percent*	*pH*		
None	418.6	100 00	2.87	0	8	493 7	100 00	2 67	4	8
Fe	12 2	2.90	3.07	0	2	18 9	3 82	2 70	0	2
Zn	91 0	21 74	2 75	0	6	234 9	47 58	2 63	0	2
Cu	367.2	87.71	2 82	0	8, br	402.7	81 57	2 76	4	4
Mn	349.8	83.55	2 85	0	8	499.3	101 13	2 44	3	4
Mo	354 3	84.64	2 88	0	8	499. 7.	101.22	2.75	3	8
Ga	358 3	85 59	2 88	0	8	506 0	102 49	2.63	2	8
Max.[3]	482.0					493 7				
C. U.[4]		19 28					21 67			
pH [5]			6 42					6 53		

[1] See table 1, footnote 2,
[2] See table 1, footnote 1,
[3] Maximum individual yield,
[4] Coefficient of utilization, or yield per 100 gm. of sucrose.
[5] Initial acidity of nutrient solution.

Requirements of *Aspergillus* were found to be somewhat different with glycerol than with sucrose. Other sources of carbon, even in minute traces, can probably cause similar variations in amino acid needs. Data to be published in another connection would indicate that minor differences may arise in "mutants" of *A. niger*, though these do not change the characteristic pattern of the normal strain. Such variations as appear can usually be associated with destructive modifications.

A distinction is necessary between results with those organisms capable and those incapable of assimilating inorganic nitrogen. Patterns of amino acid utilization will differ profoundly. Ammonia users, like *Aspergillus*, will give responses based largely on capacity for. digestion, i. e., deamination. Organisms with obligatory amino acid requirements give results based on anabolism, i. e., amination.

Response to amino acid nutrition by plants should serve to render clearer the mechanism of nitrogen storage. It is evident that those amino acids incapable of utilization when supplied from an external source are presumably unsuitable for accumulation as a nitrogen

reserve. On the basis of the responses obtained with *Aspergillus niger*, it would appear that alanine, arginine, aspartic acid, glutamic acid, glycine, hydroxyproline, ornithine, and proline may be fully reutilized as general sources of nitrogen supply. Where both nitrogen and carbon reserves are concerned the data would indicate that glutamic acid, aspartic acid, and proline would be most suitable. It is believed to be more than a coincidence that glutamic and aspartic acids are known to function in this way in the green plant in the form of their mono-amides (*15*). Availability of reserves in the seedlings important for their initial development would appear to necessitate the inclusion of carbon as well as nitrogen, since the capacity for photosynthesis is not fully developed until later.

Since interconversions of amino acids are known to take place readily, it is not strange to find investigators listing certain amino acids as alternatives. Such a procedure of course does not fully answer the question as to which is actually needed.

The degree of correlation between the results obtained on nitrogen and on carbon availability of the amino acids would strongly indicate the truth of the assumption made concerning a relation between primary origin from hexose and ammonia and rate of assimilability. Deamination or assimilation intact proceeds most readily with these first-formed amino acids, since they are in fact fully equivalent to inorganic nitrogen. Moreover, the carbon structures of these primary amino acids have not been altered appreciably from those of the original products of the oxidative degradation of hexose, to judge from their efficacy as sources of carbon. On the other hand, no real evidence was obtained that the derived amino acids are actually derived from the primary acids. It seems possible that this might be so, since the primary amino acids are fully effective as general sources of nitrogen supply. Proof of this hypothesis would require, however, that evidence be obtained that one or more of these primary amino acids is utilized without deamination.

It could not be determined whether specific quantitative trace-element requirements are associated with utilization of amino acids, nor was it possible to use this procedure in identifying unsuspected mineral requirements. Purification procedures particularly applicable to the amino acids will be necessary to permit of a repetition of these experiments at a higher level of purity. Until this has been accomplished the use of amino acids in trace-element studies should be avoided if possible.

The retention of minute quantities of the biologically essential trace elements, despite the high pH (7.0) of purification, may be found of importance in other studies. It would indicate that organic matter containing nitrogen can aid in maintaining availability of trace elements to the plant over a wide range in acidity. A similar relation may exist between the amino acids of the cell and absorbed trace elements. Amino acid complexes with heavy metals are known to exist (*11*).

Another possible explanation for the poor results on trace elements with amino acid nitrogen is the possibility of a decrease in requirements. This interpretation is unlikely if deamination is a preliminary to utilization as has been asserted. Nevertheless it cannot be eliminated without proof in the event any proportion of the amino acids are utilized as such.

SUMMARY

A survey was made of the assimilability of amino acid nitrogen and carbon with *Aspergillus niger* Van Tiegh. when grown in synthetic nutrient solutions at 35° C. for 4 days. Nitrogen was used at a level of 665 mg. per liter. Nitrogen in alanine, arginine, aspartic acid, glutamic acid, glycine, hydroxyproline, ornithine, and proline proved to be fully equivalent to inorganic nitrogen. Serine, threonine, and tryptophane gave yields corresponding to 50-percent utilization and the balance to far less. None of the amino acids fully effective as sources of nitrogen contained a stable cyclic group or branched carbon chain, nor were they among those incapable of synthesis by the rat. Assimilability of amino acid nitrogen was affected by use of a poor source of carbon supply (glycerol), though the characteristic amino acid utilization pattern was still recognizable.

Cysteine inhibited growth with both inorganic and amino acid nitrogen, and enhanced starch formation. Nitrogen utilization was very poor with cysteine, cystine, and homomethionine, and only fair with methionine. In contrast to the other sulfur-containing amino acids, homomethionine could not serve as a source of sulfur. Availability of cysteine nitrogen was not improved by oxidation of its mercapto group or replacement of mercapto hydrogen.

Only proline, aspartic acid, glutamic acid, ornithine, and arginine were at all effective as sources of carbon, the carbon utilization factors being 0.22, 0.21, 0.23, 0.10, and 0.10 respectively. These were also fully effective as sources of nitrogen. Effectiveness as sources of carbon increased synergistically in admixtures. A mixture of proline, glutamic acid, and ornithine had a carbon utilization factor of 0.82 as compared to a factor of 1.17 with sucrose. Utilization of carbon in this mixture was not increased by the addition of alanine, arginine, aspartic acid, glycine, and hydroxyproline.

Increasing length of the carbon chain in the α-amino monocarboxylic acids was accompanied by a rapid decrease in the assimilability of their nitrogen. Introduction of an amino group at terminal carbon or its oxidation to carboxyl considerably increased assimilability of both nitrogen and carbon in the four- and five-carbon acids.

Alanine, arginine (ornithine), aspartic acid, glycine, glutamic acid, and proline (hydroxyproline) are considered to be the first-formed amino acids in the synthetic process and are therefore called "primary" amino acids. These were considered probable precursors of the other amino acids synthesized by the fungus, which are termed "derived" amino acids. Carbon assimilation studies, however, indicated that α, δ-derivatives of valeric acid (glutamic acid, ornithine, proline) are actually the first of the primary acids formed, the balance being products of relatively simple interconversions.

Growth responses to structural modification in glycine and alanine when used as sources of nitrogen closely paralleled the results of analogous studies in vitro reported for enzymatic action on dipeptides. Nitrogen utilization was found directly dependent on the presence of an alpha amino group and of alpha, beta, and carboxyl hydrogen. Introduction of substituents for alpha, beta, or carboxyl hydrogen inhibited assimilability of nitrogen, as did also reduction of carboxyl or a shift of the amino group to the beta carbon atom.

The trace elements iron, zinc, copper, manganese, molybdenum, and gallium were required with amino acid nitrogen as with inorganic nitrogen. Minute quantities of these elements were tenaciously retained by the amino acids, as compared to inorganic and urea nitrogen, during purification of the nutrient solution with calcium carbonate.

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OCCURRENCE OF THE DWARF-RED CHARACTER IN UPLAND COTTON [1]

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INTRODUCTION

Red coloration in the cotton plant, due to an increase in the anthocyanin pigmentation, is a genetic character that has received wide study. For literature on the inheritance of this character the reader is referred to Carver (*1*),[3] Harland (*2, 6*), Hutchinson (*8, 10*), Mc-Lendon (*14*), and Ware (*15, 17*). Hutchinson and Silow (*11*) have listed a multiple allelomorphic series of six types of anthocyanin pigmentation in the Asiatic cottons. A similar allelomorphic series has been established by Harland (*6*) for cottons of the New World group. This latter series, however, does not include any red-plant types occurring in upland cotton, to which belong most of the types studied by investigators (*1, 13, 14, 15, 17*) in this country. Harland (*6*) states that *Gossypium hirsutum* (upland) red R_1 (*11*) is a duplicate gene to the anthocyanin allelomorphic series R_2 of the New World cottons. Hutchinson, Silow, and Stephens (*12*) have recently reported that the anthocyanin pigmentation of each of the wild species of *G. thurberia*, *G. armourianum*, and *G. aridum* belongs to the R_1 series of *G. hirsutum*. The only members of this series previously reported are red plant and normal green plant.

The dwarf character in cotton appears very infrequently as compared to the red-plant character. Harland (*3, 4, 6*) reported the occurrence of "crinkled dwarf" in sea-island cotton. He stated that this mutant is much smaller than and fully recessive to normal sea-island cotton. Crinkled dwarf was thought to occur only in sea-island cotton but has now been reported by Hutchinson and Ghose (*9*) in *Gossypium hirsutum* L. (upland cotton). In this species also the crinkled-dwarf character behaves as a complete recessive to normal. Horlacher and Killough (*7*) produced dwarf cotton plants by means of X-ray treatment of the dry seeds. As yet no inheritance studies have been reported for this type of dwarfing.

In the present paper a new type of red-plant color, together with a new type of dwarfing, in Acala cotton is described, and the results of a study of the inheritance of this character, herein designated as "dwarf-red," are presented.

DESCRIPTION OF THE DWARF-RED CHARACTER

The dwarf-red mutation of Acala cotton originated as a red chimera on a plant of normal height. It was discovered in a planting of

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foundation seed at the United States Cotton Field Station, Shafter, Calif. When first observed the mutant plant was 10 inches high, and the first red leaf was about one-half normal size. As the plant developed, the somatic variation proved to be a deep-red band comprising about one-third of the circumference of the main axis. The plant finally grew to a height of about 4 feet. As the height increased, the band of red spiraled around the axis, producing a red fruiting branch wherever it happened to coincide with a node. All nodes on the plant not intersected by the band of red produced normal green vegetation. Attempts to obtain self-pollinated seed from the red portion of the plant were unsuccessful. However, open-pollinated seed from the red fruiting branches was planted the second year to produce a progeny of 17 plants. Twelve of these plants were red throughout and the remaining five were green. The green plants had the appearance of normal Acala in every respect. The occurrence of green plants indicates that all seed produced by the chimera did not possess the factor for the dwarf-red character. The red plants varied considerably in height, but all were much shorter than the normal Acala plants. The variation in height of the red plants is further evidence of the heterozygous nature of the seed produced by the dwarf-red chimera. As will be shown later, the heterozygous dwarf-red is intermediate in height between the homozygous dwarf-red and the normal green. Self-pollinated seed was obtained from all plants of the second year except a very dwarf-red one.

The progenies of eight plants were grown the third year. Three of these progenies were from green plants and were comprised of normal green plants. The other five progenies originated from self-pollinated red plants of the second year. All segregated into three distinct types, dwarf-red, normal green, and a type intermediate in both height and red color. Of these five segregating progenies, three gave an indication of a 1 : 2 : 1 ratio. The data for this segregation are presented in table 1.

TABLE 1.—*Third-year segregation of plant lines originating from a dwarf-red chimera*

Progeny	Dwarf red	Inter-mediate	Normal green	χ^2	P
RA-3	5	13	7	0.36	0 80+
RA-5	3	11	10	4.25	.10+
RA-10	4	13	8	1 32	.50+
RA-11	2	10	12	9.00	.01+
RA-12	2	9	12	9 78	.01−
Total	16	56	49		

The monohybrid ratio obtained for three progenies is not of great significance, owing to the small numbers involved. The fact that these progenies even approached a monohybrid ratio would show that the seed produced by the chimera was heterozygous, or cross-pollinated with normal green cotton, or both. The deficiency of dwarf-red individuals would indicate that many were too weak to survive. This fact is borne out by observation, as many of this type that did survive were too weak to produce bolls. However, after the

dwarf-red was reduced to homozygosity and crossed with normal green Acala, differential viability did not occur apparently in the recovered dwarf-red segregates of the F_2. This will be noted subsequently.

Dwarf-red Acala in the homozygous condition averages 19 inches in height as contrasted with 48 inches for normal green and 32 inches for the heterozygote. Dwarfness is characterized by a decrease in the length of the internodes, as well as a decrease in the number of nodes. The homozygous dwarf-red Acala averages 23 nodes, the heterozygous dwarf-red 30 nodes, and the normal green 35 nodes per plant.

The coloring of dwarf-red Acala differs from that in other red-plant types of upland cotton. Ware (*15, 16, 17*) studied the Winesap variety of cotton and found that this variety in the homozygous condition is a deep red throughout the entire plant, stem, foliage, flowers, and bolls. In contrast to the Winesap variety, dwarf-red Acala has pale cream-colored flowers comparable to those of normal green Acala. In addition, the bolls of dwarf-red Acala are green as contrasted with the red bolls of other red-plant types of cotton. This latter observation caused R. A. Silow, who visited the Shafter, Calif., station in 1939, to express the opinion that dwarf-red Acala represents a distinctly new type of red-plant coloration in upland cotton.

The association between dwarfing and red-plant color is apparently complete; in over 1,200 homozygous red plants no exception to this rule was found. In the heterozygous condition both the color and height of plant are uniformly intermediate between the dwarf-red and normal green plants. This evident association between red-plant and dwarfism suggests linkage or pleiotropism. To date, few cases of linkage of this sort of association have been authenticated in cotton.

Chlorophyll determinations of the leaves of dwarf-red Acala, normal green Acala, as well as several other varieties of red-plant cotton, show that all have a very similar chlorophyll content. Other varieties of red-plant cotton have grown to normal height in the station nursery. This observation would largely dispel any conclusion that dwarfing was due to any local conditions of soil or climate.

INHERITANCE OF THE DWARF-RED CHARACTER IN THE CROSS DWARF-RED × NORMAL GREEN ACALA

Homozygous dwarf-red Acala when crossed with normal green produced in the F_1 generation a progeny that was uniformly intermediate in both color and height of plant between the two parental types. As mentioned above, the "crinkled dwarfs" reported by Harland (*4, 5*) and Hutchinson and Ghose (*9*) were entirely recessive in the F_1 or heterozygous condition. However, the intermediate red coloration in the dwarf-red heterozygote agrees with such studies previously reported (*1, 6, 8, 14, 15, 17*). The F_2 generation segregated into the monohybrid ratio of 1:2:1. Table 2 presents the data of this segregation along with χ^2 for goodness of fit to a 1:2:1 ratio. Corresponding P values indicate a satisfactory agreement between the theoretical 1:2:1 ratio and observation.

TABLE 2.—*Segregation of the F_2 generation in the cross between dwarf-red Acala and normal green Acala*

Progeny	Dwarf red	Inter-mediate	Normal green	χ^2	P
93	88	155	99	3.70	0.20−
10–20	6	12	6	.00	1.00
10–21	6	15	3	2.25	.30+
21–1	19	28	19	1.52	.50−
21–11	28	47	26	.56	.80−
20–15	14	32	14	.27	.90−
20–22	18	42	17	.66	.70+
20–24	20	32	16	.71	.70+
20–1	20	41	20	.01	.99+
20–10	18	38	15	.61	.70+
20–13	36	44	23	5.47	.05+
6–2	8	16	9	.09	.95+
Total	281	502	267		..

A backcross of the heterozygous dwarf-red to either parental type produces in the following generation a monohybrid ratio of 1 hetero-zygote to 1 parental type. Table 3 reports the results of the backcross between a heterozygous dwarf-red and normal green. Chi-square and the corresponding P values indicate a very satisfactory agreement between the observed and the theoretical 1 : 1 ratio. The backcross to the other parental type, dwarf-red, resulted in 23 intermediate red plants and 25 normal dwarf-red plants. As will be readily seen, this also is a very satisfactory agreement with the theoretical 1 : 1 ratio.

TABLE 3.—*Segregation of the backcross generation in the cross between the F_1 and the normal green Acala*

Progeny	Intermedi-ate red	Normal green	χ^2	P
3–2	26	32	0.62	0.50−
4–2	17	16	.03	.90−
51	13	10	.39	.50+
40	9	7	.25	.70−
Total	65	65	...	...

SUMMARY

The mutation of Acala cotton herein designated as "dwarf-red" originated as a chimera on an otherwise normal green plant.

Seeds produced by the chimera were heterozygous for the dwarf-red character.

A new type of red-plant color in upland cotton is described.

A new type of dwarfing in cotton is described which is not recessive in the heterozygous condition.

Dwarfing in this type is closely associated with red-plant color. Possibly both expressions are controlled by the same factor.

Dwarf-red Acala when crossed with normal green Acala produced an F_2 generation that was intermediate in both color and plant height between the parental types.

The F_2 generation of the cross between dwarf-red Acala and normal green Acala segregated into the 1 : 2 : 1 ratio, inducing a simple monohybrid.

Likewise the backcross generations obtained from the crosses made between the F_1 or the heterozygous dwarf-red and the parental

types verified the monohybrid condition, both segregating into the
1:1 ratio.

Indications are that dwarf-red is controlled by a single factor.

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THE ASCORBIC ACID CONTENT OF DIFFERENT VARIE-TIES OF MAINE-GROWN TOMATOES AND CABBAGES AS INFLUENCED BY LOCALITY, SEASON, AND STAGE OF MATURITY [1]

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INTRODUCTION

In 1935, studies of food production by Dove (6) [3] indicated that dietary vitamin C was lacking in various parts of Maine. The soundness of this observation has been confirmed in at least one section of the State by a clinical and dietary study of vitamin C nutrition in school children (4, 14).

The determination of the vitamin C content of Maine-grown foods has therefore become an important part of the experimental nutritional program of the Maine Agricultural Experiment Station (15). This program involves the investigation of many of the determining factors which affect the vitamin C content of fruits and vegetables. Of these factors, differences concomitant with variety are of extreme importance. The effects of maturation, distribution of the vitamin within the plant, soil type, fertilizer treatment, and geographical situation are also being observed.

Beacham and Bonney (1) found that Valencia oranges (from sour orange rootstock) grown in three localities in Florida varied in ascorbic acid content from 0.36 to 0.63 mg. per cubic centimeter of orange juice. The variations within each locality were nearly as large: in Orlando, from 0.44 to 0.63 mg. per cubic centimeter; in Mims, from 0.40 to 0.60 mg. per cubic centimeter; and in Wabasso, from 0.36 to 0.60 mg. per cubic centimeter. French and Abbott (7) found no correlation between the ascorbic acid content of oranges and the location in which they were grown. Metcalfe, Rehm, and Winters (12) observed inconsistent locality variations in oranges and grapefruit and attributed the differences to variations in soil.

To determine the effects of geographical situation on the production of ascorbic acid in plants, three experiments were carried out, one in 1938 and two in 1939.

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EXPERIMENT WITH TOMATOES, 1938

PROCEDURE

Four varieties of tomatoes (*Lycopersicum esculentum* Mill) were chosen to be grown in four widely different sections of the State of Maine. The localities selected were Aroostook Farm, at Presque Isle, in the northern part of the State; Orono in the central part; Highmoor Farm, at Monmouth, which is rather more southern; and Kennebunk, which is not far from the southern extremity of the State.

The varieties chosen were Penn State Earliana, Bestal, Best of All, and Comet. The two first named, from preliminary tests, appeared to be relatively low in ascorbic acid content; the other two were at the upper end of the scale. By selecting these extremes, any variations due to geographic situation within this large State would soon become apparent.

Because of the many factors that may occasion differences in ascorbic acid content, the data shown in table 1 were recorded for the four localities in which the plants were grown.

The plants were started from seeds which were planted in sterile sand April 23, 1938. On May 16 the seedlings were transferred into bands in plots of soil. The young plants were set outdoors on the following dates: At Aroostook on June 17; Orono, June 4; Highmoor, June 10; and Kennebunk, June 3.

Testing was begun August 11, and the tomatoes were analyzed at weekly intervals thereafter for a period of 6 weeks. The plants grew and fruited at different rates in the different localities. The weather conditions during the summer of 1938 were not auspicious for tomato ripening in Aroostook, but in the southern sections they were particularly conducive to good growth and development. This fact made for a diversity among the samples that was undesirable. Thus, although conditions were controlled as completely as possible, the samples were not strictly comparable at all times.

TABLE 1.—*Soil type, latitude, and length of growing season at the 4 localities in which tomatoes were grown*

Locality	Soil type	Latitude	Length of growing season (11)
			Days
Aroostook	Caribou loam	46°40′30″	90 110
Orono	Silty clay loam	44°54′2″	120-130
Highmoor	Heavy loam	44°14′24″	140-150
Kennebunk	Adams sandy loam and fine sandy loam	43°24′	160-170

Even green tomatoes were not available from Aroostook at the first testing date. At Highmoor and Kennebunk, the fruit matured early and samples were not obtainable for the later testing dates. Not more than 10 ripe tomatoes were harvested in Aroostook, making it necessary to compare green with ripe tomatoes after the third testing date. Through an oversight, the Bestal variety was not included at Highmoor so that the data for that section were not complete.

The material to be tested was harvested in each of the four sections on the same day at approximately the same time.   The analyses were made at Orono.   The tomatoes were sent by express from the other localities and arrived usually about 12 hours after they were harvested. The Orono samples were kept at room temperature until the other samples arrived.   All the samples were analyzed at the same time.

A composite sample to weigh between 20 and 30 gm. was made up of radial sectors from each of four tomatoes.   Duplicate samples consisted of the opposite radial sectors from the same tomatoes. The ascorbic acid content was determined by the titrimetric method developed by Bessey and King (*3*) as later modified by Musulin and King (*16*).   Three percent metaphosphoric acid was used as an extractant according to recommendations by Bessey (*2*).   The results of the analyses are presented in figure 1.

EFFECT OF LOCALITY

It may be seen that, with the exception of one variety, all the tomatoes from Aroostook were lower in ascorbic acid content than those from the other localities.   The Aroostook-grown Bestal tomatoes manifested higher ascorbic acid values than those from Orono for 2 weeks.   Perhaps it is significant that at the testing dates when the Aroostook Bestal tomatoes were at the highest peak, all the sample tomatoes of that variety were ripe.   Maclinn, Fellers, and Buck (*9*) found no consistent increase in the vitamin C content of tomatoes during three stages of maturity.   In order to determine the effect of ripeness at Orono, 10 pairs of samples of tomatoes including 7 varieties were analyzed.   Both green and ripe tomatoes from the same plants were tested on the same day.   In every case the ripe tomatoes were higher in ascorbic acid than the green.   The mean difference was 0.09 ± 0.01 mg. per gram with infinite odds that the difference was significant.

The variations depicted in figure 1 may have been due to a number of influences.   Although there is a difference of over 3° in latitude between Aroostook and Kennebunk, this seems not to have been the factor that caused the vitamin C differences since there is half that latitude difference between Orono and Kennebunk and the vitamin C values show no variations.

Killing frosts are common much later in the spring at Aroostook than at the other three localities.   This necessitates setting plants out at a later date and, in consequence, the tissue tested at any given time is less mature than that from plants grown at the more southern localities.   Although it has been shown that the green tomatoes from a given variety have lower ascorbic acid values than the ripe fruit, it is obvious from the graphs that the differences observed during this experiment were not due entirely to the immaturity of the tissue.   At the first three testing periods all the tomatoes were green and the smaller content of ascorbic acid in the Aroostook samples was even then apparent.

Data on weather conditions for the four localities under investigation were incomplete.   For Kennebunk, it was necessary to use the data accumulated at Portland, Maine, which is approximately 30 miles northeast of Kennebunk.   Both, however, are coastal towns and weather conditions are similar.   For Highmoor, the data accumulated

at Lewiston (12 miles southwest) were used.   For Orono and Aroostook the weather data were recorded at the locality in which the crops were grown.
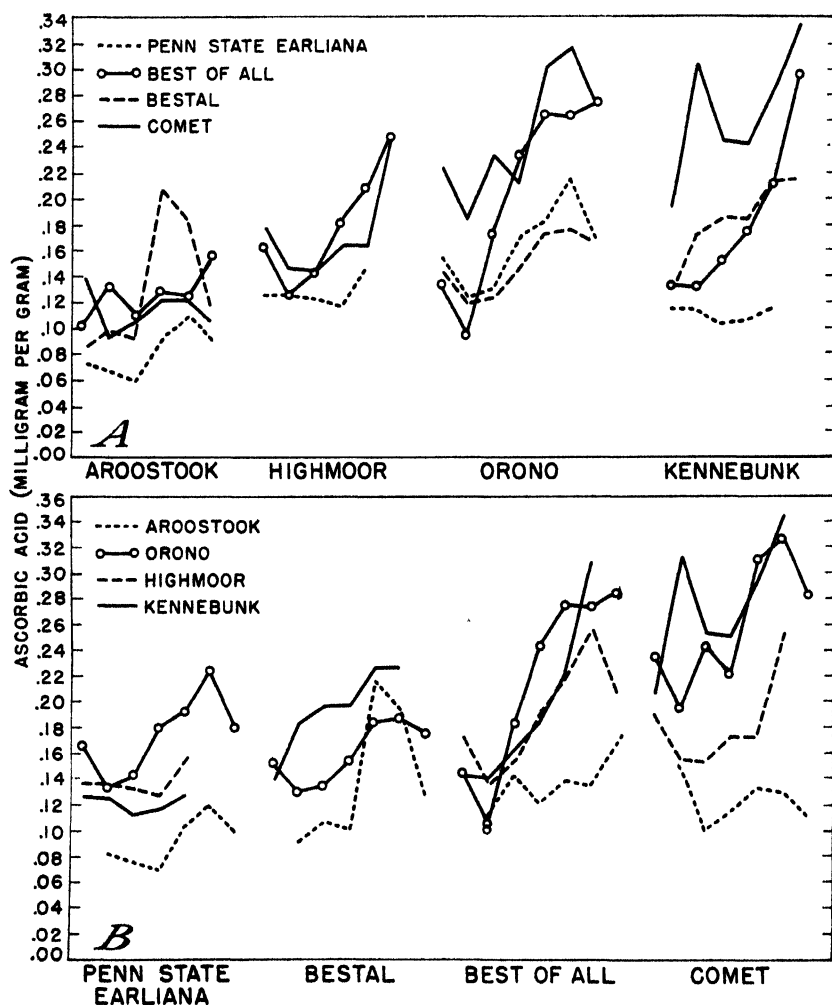
FIGURE 1.—The ascorbic acid content of tomato fruit grown in four different localities in Maine during the summer of 1938: *A*, Amounts of ascorbic acid arranged according to locality in which tomatoes were grown; *B*, amounts arranged according to variety.   Determinations were made at weekly intervals and are shown in chronologica, order.

The average temperature during the growing season of 1938 in Aroostook was somewhat lower than in the other places: for Aroostook 60.6° F.; for Orono, 66.8°; for Highmoor, 68.0°; and for Kennebunk, 66.6°.   It seems unlikely that these differences were large enough to

be the chief factor causing the ascorbic acid variations. If the temperature had been low enough to inhibit metabolic processes, the view that low temperature was responsible for low ascorbic acid would be more tenable.

<div align="center">EFFECT OF VARIETY</div>

Varietal differences (fig. 1) were not as consistent as preliminary experiments had indicated. Originally fairly large differences were apparent between the Comet and the Penn State Earliana varieties when the plants were grown outdoors. The differences were smaller when the plants were grown in the greenhouse. The reverse appeared to be true of the Best of All and the Bestal varieties. The differences between these two were large when they were grown indoors, but smaller when they were grown outdoors.[4]

In this experiment the Penn State Earliana tomatoes exhibited the smallest values for vitamin C of the four varieties in all sections except Orono, where it showed a larger average value than Bestal. Bestal was the most variable of the varieties in respect to vitamin C, its values ranging from 0.09 to 0.21 mg. per gram in Aroostook alone. In Aroostook, the Bestal had the highest average value (0.14 mg. per gram) of the four varieties. The average values for Bestal and Best of All were exactly the same (0.20 mg. per gram) at Kennebunk. Bestal showed a lower value than Comet and Best of All at Orono.

The foregoing data suggested a trend which called for further investigation to determine whether vitamin C values are consistently on a lower level in Aroostook or whether the data designated a spurious phenomenon peculiar to the season of 1938. Should the former prove to be the case, it would be necessary to obtain varieties which are early, adaptable, and rich in vitamin C when grown locally to provide this large northern section of the State with at least a part of its supply of this essential vitamin.

<div align="center">EXPERIMENT WITH TOMATOES, 1939</div>

In order to increase the reliability of the observations made during the season of 1938 on differences in the vitamin C content of tomatoes coincident with geographical situation, the experiment was repeated during the summer of 1939.

Although no ripe tomatoes had been available from Aroostook during the 1938 testing season, it was deemed advisable to adhere to the experimental procedure as originally planned rather than to add new variables by changing the varieties to be observed.

<div align="center">PROCEDURE</div>

The plants were set out on the same locations as were used in 1938 so that the data recorded above as regards soil type, latitude, and length of growing season are applicable to the second season's experimentation. The soils for the four plots were analyzed and the data are recorded in table 2.[5] The young plants were set outdoors on the

following dates: At Aroostook, June 14, 1939; Orono, June 6 and 7; Highmoor, June 8; and Kennebunk, June 6.

Testing was begun on August 10, approximately the same date as in the previous season. Subsequent determinations were made weekly until September 21. Cultural conditions were similar to those of the preceding year. The method of sampling was identical with that outlined for the 1938 experiment.

TABLE 2.—*Analytic data for soils in the experimental plots at the four localities in which tomatoes were grown in 1939*

Locality	Reaction (pH)	Nitrates	Ammonia	Phosphorus	Potassium	Calcium	Magnesium	Manganese	Iron	Aluminum
Aroostook	[1] 6 29	[2] 100	[3] 0+	[4] 50+	[5] 100	[6]	[2] 400	[5] 25−	[3] 5−	
Orono	6. 00	[6]	0+	200	(6)	2,000	100+	10−	5+	250−
Highmoor	5 67	(6)	400	200−	(6)	1,500	100−	10−	5	250−
Kennebunk	5 01	(6)	200−	300	(6)	3,000	200	25+	5	100

[1] Moderate.
[2] Very high
[3] Trace

[4] Medium low.
[5] Low
[6] Excess.

EFFECT OF LOCALITY

At the end of three testing periods it was obvious that history was repeating itself only insofar as to show that during any one season, locality had a marked effect upon ascorbic acid production. During these three periods, the tomatoes from Aroostook were found to contain nearly as much ascorbic acid as those from Orono, while the samples from the two more southern localities contained much less than those from Orono and Aroostook. The amounts of ascorbic acid in the samples for the entire period are shown graphically in figure 2.

Soil type and fertilizer treatment have been reported as playing a part in the production of ascorbic acid by plants (19), although later observations do not confirm this finding (10).

Soil analyses were not made during the 1938 investigation. The soil analytic data for the 1939 experiment were examined on the presumption that differences in available soil nutrients may have caused the variations in ascorbic acid content. Insofar as soil analyses reveal the quality of the soil as regards those elements now known to be essential, it was judged that neither soil type nor available nutrients were responsible for the ascorbic acid variations in this experiment. This does not preclude the possibility that the soil condition may be found to be a determining factor in vitamin C synthesis in the light of future advances in soil science.

The possibility of a loss through oxidation of the ascorbic acid between the times of harvesting and testing was suggested. Although the tomatoes were harvested at approximately the same times and held at room temperature until analysis was begun, there was a possibility that shipping conditions from Kennebunk and Highmoor were of such a nature as to destroy vitamin C. High temperatures would be the main condition that might bring about this destruction. Although Maclinn, Fellers, and Buck (9) found no diminution in the ascorbic acid content of tomatoes stored at room temperature up to

240 hours, it was possible that the experimental conditions in Maine were not exactly analogous to those in Massachusetts.

Accordingly it was arranged to have the tomatoes shipped in containers with solid carbon dioxide whereby the temperatures were kept near or below freezing. The samples were shipped in this way for three testing periods. The ascorbic acid values of these tomatoes are recorded in table 3.

FIGURE 2.—The ascorbic acid content of tomato fruit grown in four different localities in Maine during the summer of 1939: *A*, amounts of ascorbic acid arranged according to locality in which tomatoes were grown; *B*, amounts arranged according to variety. Determinations were made at weekly intervals and are shown in chronological order.

TABLE 3.—*Effect of low temperatures during shipping on the ascorbic acid content of tomatoes*

[Four varieties grown at 4 localities in Maine]

Variety	Number of analyses and shipping conditions	Aroostook	Orono	Highmoor	Kennebunk
		Mg. per gm.	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*
Penn State Earliana.	3 analyses; with solid carbon dioxide.	0.18	0.21	0.15	0.12
	4 analyses; without solid carbon dioxide.	.17	.19	.13	.10
	7 analyses; with and without solid carbon dioxide	17	20	.14	.11
Comet.	3 analyses; with solid carbon dioxide.	.22	33	.18	.16
	4 analyses; without solid carbon dioxide.	.20	.26	.16	.15
	7 analyses; with and without solid carbon dioxide.	.21	29	16	15
Bestal.	3 analyses: with solid carbon dioxide.	19	27	.18	.13
	4 analyses; without solid carbon dioxide.	.20	22	14	.13
	7 analyses; with and without solid carbon dioxide.	19	21	.16	.13
Best of All.	3 analyses; with solid carbon dioxide.	.22	30	.21	.14
	4 analyses, without solid carbon dioxide.	.21	.24	.19	.12
	7 analyses· with and without solid carbon dioxide.	.21	.26	.20	.13

It will be noted that the figures for Kennebunk and Highmoor are proportionately the same when compared to those for Aroostook and Orono as they were before the precaution of icing was taken. They are comparable to the values obtained while the samples were shipped at the existing temperatures or to the average of all the determinations including those from fruits shipped with and without ice. The slightly higher figures obtained for tomatoes shipped in ice were caused by the fact that during these periods practically all of the tomatoes were ripe, whereas two or three of the four samples averaged for the groups shipped without ice consisted of green tomatoes. Hence an effect of maturity is manifested in these higher values. Loss of vitamin content through oxidation caused by high temperatures apparently was not the cause of the locality variations. The tomatoes analyzed on the last testing date were not shipped in ice.

EFFECT OF VARIETY

Varietal differences were similar to though less variable than those observed in 1938. The data are shown in figure 2. In all four localities the Penn State Earliana variety had the lowest values for ascorbic acid content. Bestal was slightly higher than Earliana in all localities and was consistently the second lowest. Comet was highest at Orono and Kennebunk, whereas Best of All was highest at Aroostook and Highmoor. The values for these two latter varieties were quite similar so far as variety is concerned, although variations within variety caused by geographical situation were comparatively large.

EFFECT OF MATURITY

Again, in the season of 1939 increases in ascorbic acid coincident with maturation were evident. A gradual rise with the advent of

ripeness was noted, with more or less constant values apparent after complete ripeness was attained. The effect of overripeness was not determined. The averages for the green and the ripe tomatoes are listed in table 4. It is interesting to note that while five of the seven samples tested from Aroostook consisted of green tissue, the values are still higher than those of Highmoor and Kennebunk where five of the seven samples were ripe tomatoes. Locality differences are large enough to overshadow the effects of maturity.

EFFECT OF WEATHER CONDITIONS

Weather conditions were better in Aroostook in 1939 than in 1938. In spite of this, ripe tomatoes were available for only three of the testing dates, whereas five samples of ripe fruit were available from the other localities. At Kennebunk and Highmoor, there were few clear sunny days. Especially at Kennebunk, heavy fog prevailed over long periods. Excellent fruit was shipped in, however, from the two southern localities, and the local growers considered 1939 to be a good tomato season.

The available weather data were inadequate to establish a premise on the basis of meteorological facts. The amount of sunshine is not recorded in Orono or Aroostook except in terms of clear, cloudy, and partly cloudy days. It was possible to calculate the hours of sunshine from the Lewiston records, and the percentage of possible sunshine was recorded at Portland. Temperature and rainfall data were accurately recorded in all four localities.

TABLE 4.— *Effect of maturity on ascorbic acid content of 4 varieties of tomatoes grown at 4 different localities in Maine in 1939*

Variety	Condition of fruit	Ascorbic acid content of tomatoes from—			
		Aroostook	Orono	Highmoor	Kennebunk
		Mg. per gm.	*Mg. per gm.*	*Mg. per gm.*	*Mg. per gm.*
Penn State Earliana	{Green	0.15	0.18	0.10	0 09
	{Ripe	22	.21	.15	.12
Comet	{Green	.19	.21	12	12
	{Ripe	.25	32	18	.17
Bestal	{Green	.19	20	11	.11
	{Ripe	.20	.25	19	.14
Best of All	{Green	.19	.19	14	.12
	{Ripe	.23	.29	22	.14

It has been shown by Kohman and Porter (8) that the solar rays impinging upon tomato plants immediately before testing greatly affect the ascorbic acid content. These workers stated that when tomato plants were taken from the outdoor sunshine into the greenhouse, a marked drop in vitamin C resulted. Reid and Weintraub (18) and Reid (17) observed an increased synthesis of ascorbic acid when excised moonflower roots and young cowpea plants were grown in light instead of darkness. Currence (5) found no significant correlation between the vitamin C of tomato plants and the percentage of sunshine. His plants were grown in a greenhouse, however, where environmental conditions are not comparable to those of the outdoors (8). Furthermore, all of his reported values were relatively low, ranging from 0.09 to 0.14 mg. per gram. Varieties with high

vitamin C values may emphasize a relationship to light if one exists. Currence's data were taken during November, December, and January when even a high percentage of possible sunshine may not have been favorable for maximum vitamin C production. Reid [6] noted that roots with a low content of ascorbic acid during a prolonged cloudy period showed a marked increase of this substance following a day of bright sunshine. Moldtmann (*13*) pointed out that a lowering of the vitamin C content occurs also in the leaves of *Fagus sylvatica* during periods of cloudy weather, while an increase is apparent during sunny weather. These observations indicate that the environmental conditions to which the plant is exposed immediately prior to analysis have a disproportionate influence on ascorbic acid synthesis in relation to conditions which exist during a complete season. Therefore, averages of weather data were calculated for the growing season (from planting date until date of last harvest), for the testing season, for 3 days before each harvest, and for the day of harvest. The available data are presented in table 5.

TABLE 5.—*Temperature, rainfall, and sunshine at the four Maine localities in which tomatoes were grown during the summers of 1938 and 1939*

Locality	Year	Average temperature		Rainfall				Sunshine			
								Total, 3 days before harvest		Total, day of harvest	
		Growing season [1]	Testing season [1]	Growing season [1]	Testing season [1]	Total, 3 days before harvest	Total, day of harvest	Hours of sunshine [2]	Percent of possible sunshine	Hours of sunshine [2]	Percent of possible sunshine
		F.	*F*	*Inches*	*Inches*	*Inches*	*Inch*	*Hours*	*Percent*	*Hours*	*Percent*
Aroostook	1938	60 6	56 1	15 72	6 49	2 57	0 70				
	1939	64 4	63 8	13 80	4 32	2 05	96				
Orono	1938	66 8	63 2	11 84	5 23	1 12	.76				
	1939	66 2	65 8	9 60	2 22	2 45	24				
Highmoor [3]	1938	68 0	63 2	15 00	5 33	1 84	12	159 5		45 5	
	1939	68 1	68 1	9.60	1 63	3 07	.43	117 2		44 0	
Kennebunk [4]	1938	66 6	67 9	17.40	2 06	03	01		91		75
	1939	65 9	66 5	11 31	2 49	2 95	37		57		70

[1] The growing season was from planting date to date of last harvest, the testing season from first to last harvest.
[2] The writer is indebted to Paul A. Bean, of Lewiston, Maine, for the sunshine records.
[3] Lewiston.
[4] Portland.

An analysis of the data shows that at Aroostook in 1938, when vitamin C values were low, rainfall during the growing season amounted to 15.72 inches, whereas in 1939, when vitamin C values were higher, rainfall was 13.80 inches. For the 43 days of the testing period, the 1938 rainfall was 6.49 inches and the 1939 rainfall 4.32 inches—a decrease of 33 percent. Less difference was observed between the averages of the 3 days before harvests, and the 1939 rainfall was higher when harvest dates alone are considered. The mean temperature was 3.8° lower in 1938 during the growing period and 7.7° lower during the testing period. There were 12 clear days and 28 cloudy

[6] REID, MARY ELIZABETH. Personal communication, 1941.

or partly cloudy ones during the 1938 testing period as compared with 17 clear days and 25 cloudy ones during 1939.

Orono conditions were similar for both seasons with somewhat less rainfall in 1939.

At Highmoor and Kennebunk the rainfall was less in 1939 during both growing and testing periods. In 1939 there was about double the amount of rainfall at Highmoor when the 3-day periods prior to harvest were totaled and at Kennebunk the increase was very large.

The records for available sunshine were fairly adequate for Lewiston and Portland, and these tend to uphold the premise that sunshine is one of the chief factors contributing to the production of ascorbic acid by the plant. The variations are striking during the 3-day periods.

EXPERIMENTS WITH CABBAGE, 1939

To obviate drawing conclusions from results limited to one vegetable whose reactions to environmental factors might possibly be unique, an analogous experiment with another crop was planned and executed in 1939. Cabbage (*Brassica oleracea L*.var. *capitata L.*) was chosen for this parallel experiment, since it can be grown to maturity in all parts of Maine and is known to be an excellent source of vitamin C.

Four varieties, each representing a type, were planted in adjacent plots in the four localities. Soil analytic data were similar to those for the tomato plots. The varieties planted were as follows:

Variety	*Type represented*
Cornell Early Savoy	Savoy.
Yellows Resistant Copenhagen	Copenhagen Market.
Jersey Wakefield	Wakefield.
Penn State Ballhead	Danish Ballhead.

The first three varieties mature during the latter part of July and the first part of August. The Penn State Ballhead is in its prime about the first of October, when the other varieties are either unavailable or, at least, are definitely past the peak of their development.

The cabbage seed was treated with Semesan and planted in sand on May 2, 1939, and the young seedlings were transferred to soil on May 18. The plants were transported to the field and set out on the following dates: At Aroostook on June 14; Orono, June 6 and 7; Highmoor, June 15; and Kennebunk, June 16.

Once weekly for 3 weeks the young plants were treated with mercuric chloride solution to prevent maggot injury.

Because of the differences in time necessary to attain maturity, testing was started early in the season and carried out through October when the Ballhead variety became mature. The plants were shipped by express to the Orono laboratory and were analyzed immediately upon arrival. Similar conditions of harvesting and shipping were maintained with the cabbages as with the tomatoes, including the interval when solid carbon dioxide was used to control the temperature during shipment. No losses ascribable to shipping conditions were observed.

Testing was begun July 19, 1939, and subsequent determinations were made at biweekly intervals until October 11. During July, the plants were large but the heads were just beginning to form. The plants from Aroostook were smaller than those from the more southern

localities. During the latter part of the season, the earlier varieties were not available at all of the localities. Those that could be procured are included in the report if at least two places are represented.

The sampling procedure presented difficulties. It was desired to keep the samples relatively small so that dissolution of the vitamin in the extractant would be complete. On the other hand, differences in vitamin C values among analogous cabbages as well as uneven distribution of the vitamin within individual plants made it imperative to use several vegetables for a composite sample in order to have the sample truly representative of the variety.

A very thin cross section was taken through the whole cabbage, cutting as near as possible at the center of the head. When the plants were young and immature, this region was very near the base stalk. As the head grew larger, the sample was taken farther away from the base. None of the hard main stalk was included in the sample, but
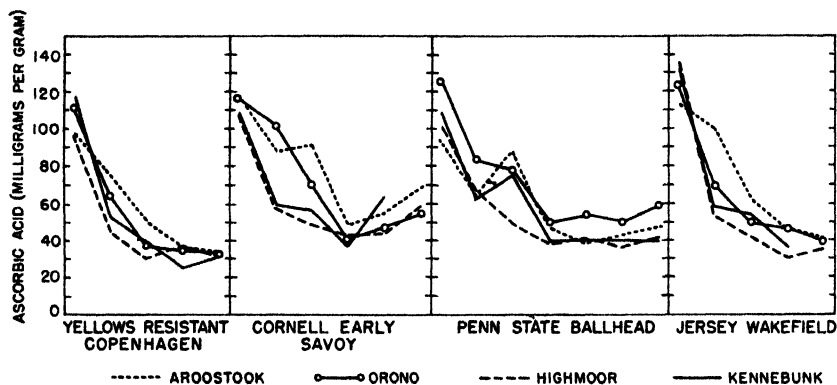
FIGURE 3.—The ascorbic acid content of four varieties of cabbage grown in four different localities in Maine during the summer of 1939. Determinations were made at biweekly intervals and are shown in chronological order.

the smaller stalks of the large, individual outer leaves were sectioned along with the rest of the cabbage. Composite samples consisted of these very thin sections from three plants whenever possible. As a result of excessively heavy rains in Aroostook some of the plants were washed away, leaving a shortage of test material. Therefore, it was necessary occasionally to use two plants and sometimes only one plant for a sample. Duplicate samples consisted of thin slices taken just above the original samples.

The results of the analyses are shown graphically in figure 3. The effects of maturity are worthy of note. When the plants were young and the tissue most highly active metabolically, the ascorbic acid values were extremely high, but with the approach of maturity they dropped rapidly. This condition is consonant with the view that vitamin C has a role to play in metabolism or is a byproduct thereof. The same diminution in ascorbic acid with the onset of maturity has been observed in onions and apples.[7] The converse seems to be true of tomatoes, i. e., as maturity is achieved, the ascorbic acid content increases. The sharp break in the curves demonstrating the values

[7] MURPHY, E. F. Unpublished data, 1940.

of Cornell Early Savoy cabbage at the end of the sixth week and a subsequent upward swing are worthy of note.   A lack of test material prevented further investigation of this increase.

With but slight variations, all four varieties (fig. 3) show remarkable similarities in respect to vitamin content.   A distinct trend in ascorbic acid decline as the plants approached maturity is clearly apparent for all four varieties in all four localities.   Although the values are not plotted on a semilogarithmic scale, the manner in which the varietal lines bunch together on the arithmetic scale with narrow intervals indicates that the ascorbic acid per gram of cabbage of the four varieties not only decreases by similar absolute amounts with time but, as indicated by the similar slopes of the lines, decreases at approximately the same rate.   The slight differences in rate of diminution for the different localities is not enough to erase the more favorable results in Aroostook and Orono.   The rapid rate of decline, indicated by the steep slopes of the lines based on the earlier tests, could very well intensify the differences in favor of Aroostook and Orono because of the relative physiological immaturity of the plants from these regions. This variable would lose its importance, however, as all the plants become mature and all the curves level off, or even, as in the case of the Cornell Early Savoy, actually turn up.   This variable might be eliminated from the charts by shifting the Orono and Aroostook curves to the left, but this would necessitate an arbitrary assumption as to the physiological age of the tissue.   It was thought best not to do this, but caution is urged against a spurious interpretation from the middle sections of the charts.   The leveled sections of the charts, when physiological age differences are presumably minimized, show a higher vitamin content in Aroostook and Orono in the majority of cases, and this is in essential agreement with the results of the tomato experiments discussed above.

DISCUSSION

The variations in ascorbic acid noted in food crops grown in different locations are shown here to be undoubtedly caused by environmental agents and therefore are seasonal rather than locality variations.   To demonstrate the effects observed, the differences between each locality and every other locality were plotted.   Two of these graphs are included.   Figure 4 shows the variations in the vitamin C content of tomatoes grown in Aroostook as compared with Orono, Highmoor, and Kennebunk in 1938 and 1939.   The dissimilarities of the charts for the 2 years are striking.   In 1938 the ascorbic acid of Aroostook tomatoes was lower at all testing periods than that of tomatoes grown in the other three localities.   In 1939 the values for Aroostook tomatoes were lower than those from Orono, somewhat higher than those from Highmoor, and markedly higher than those from Kennebunk.   That this is a seasonal effect is apparent from figure 5, in which the differences between varieties in each locality for 1939 and 1938 are compared.

The differences between the ascorbic acid values of cabbages grown in Aroostook in 1939 and the same varieties grown in the other three localities are depicted in figure 6.   The Aroostook-grown cabbage shows a higher ascorbic acid content per gram than those produced

in Orono, Highmoor, and Kennebunk for all varieties and for practically all testing periods except the first. It may be that the production of vitamin C increases from the time the plants are set out until the cells are no longer highly active in a metabolic sense and are substituting growth in size for cell division. If this assumption is correct, the peak may well have occurred in cabbages at the first testing date in Orono, Highmoor, and Kennebunk, whereas it may not have been attained in Aroostook until 2 weeks later, when the shift in the opposite direction is very marked. Thus a developmental may be superimposed upon a seasonal influence to increase the complexity of interpretation.
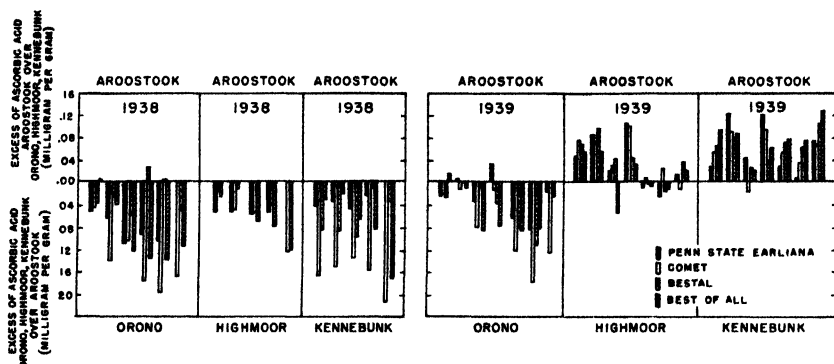
FIGURE 4.—The differences between the ascorbic acid values of four varieties of tomato grown in Aroostook and the same varieties grown in three other Maine localities. Each rectangle represents the ascorbic acid difference between tomatoes of one variety grown in two localities. Each group of rectangles represents one testing period, and the groups are arranged in chronological order of testing periods. Zero is an assumed point of uniformity at which there would be no locality variations.

TABLE 6.-- *Variations in the ascorbic acid content of tomatoes as affected by environment and variety*

Successive weekly tests during fruiting period [1]	Environmental differences Average differences in vitamin C between the same varieties grown in 4 localities	Varietal differences Average differences in vitamin C between unlike varieties in each of 4 localities	Difference in favor of environmental effect
	Mg. per gm.	*Mg. per gm.*	*Mg. per gm.*
1	0 037±.0064	0.023±.0052	0.014
2	052± 0065	.035±.0058	.017
3	.041±.0048	.037±.0036	.004
4	.060± 0065	.034± 0040	.026
5	.067±.0071	.044±.0051	.023
6	084± 0091	.035±.0035	.049
7	.077±.0082	.045±.0087	.032
Mean deviation for 7 periods	.060±.0029	.036±.0018	.024±.0034
Standard deviation	0383	.0246	

[1] $N=12$ comparisons (4 varieties or 4 localities taken 2 at a time).

In table 6 the variations imposed by environment are compared to those inherent in the plants. The environmental difference shown for each testing period is the average of the differences in ascorbic acid
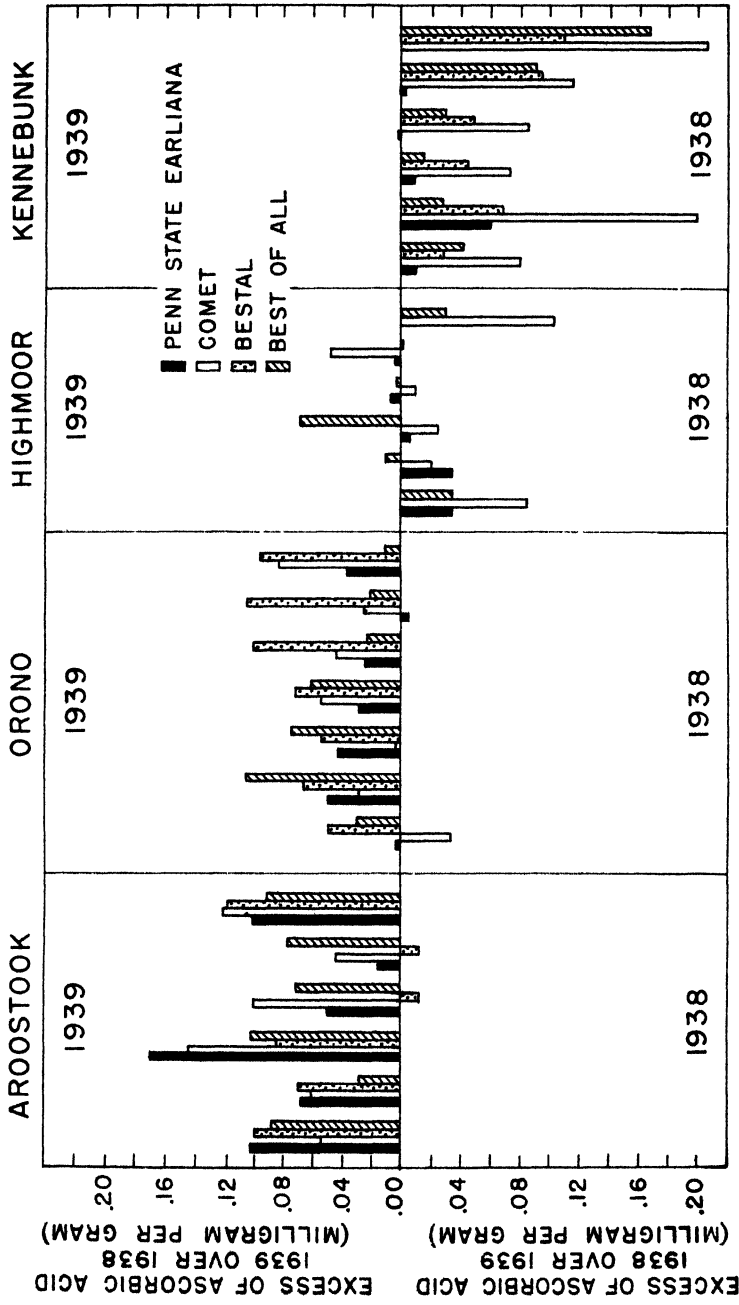
FIGURE 5.—Seasonal deviations in the ascorbic acid content of four varieties of tomato grown in four localities in Maine. The tomatoes were produced on the same plots each year. Each rectangle represents the ascorbic acid difference between 1939 and 1938 for one variety of tomato. Each group of four rectangles is arranged in chronological order of testing periods.

content between the values for the same varieties in four localities. The varietal difference for each testing period is the average of the differences in ascorbic acid content between the values in the same localities of four varieties. In the fourth column the values expressing the effect of environment are shown to be in excess of those expressing the effect of variety in all seven testing periods.

Currence (5) points out that environmental effects vitiate the significance of varietal difference, and that recommendations to growers emphasizing the nutritional superiority of any particular variety are misleading. In these experiments, the amounts of ascorbic
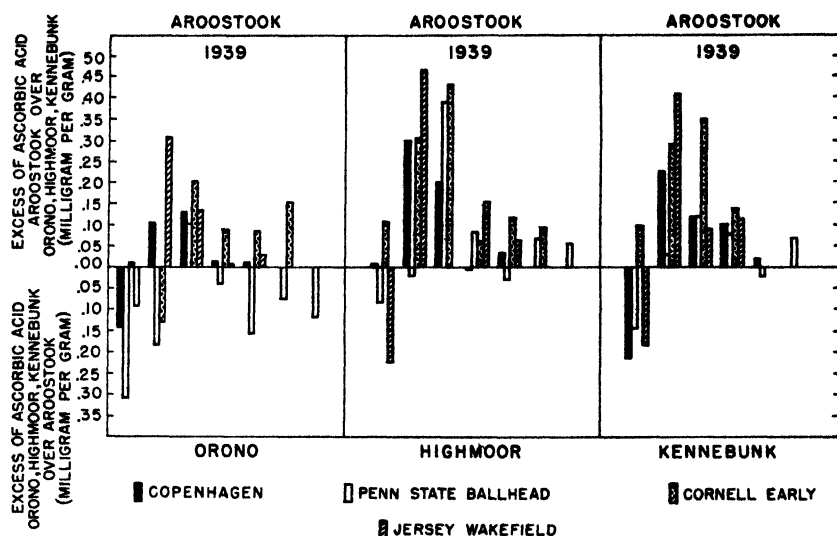
FIGURE 6.—The differences between the ascorbic acid values of four varieties of cabbage grown in Aroostook and the same varieties grown in three other Maine localities. Each rectangle represents the ascorbic acid difference between cabbages of one variety grown in two localities. Each group of rectangles represents one testing period, and the groups are arranged in chronological order of testing periods. Zero is an assumed point of uniformity at which there would be no locality variations.

acid coincident with variety as depicted in figures 1 and 2 are worthy of note. In spite of the magnitude of the variations caused by environmental agencies, varieties having high ascorbic acid values remain in a relatively high position even when grown under adverse environmental conditions, e. g., the Comet (a high variety) is significantly higher than Penn State Earliana (a low variety) in Kennebunk during 1939, although it is lower than Penn State Earliana grown in Aroostook in the same season, whereas Comet in Aroostook is significantly higher than Earliana in that locality. The mean differences of these comparisons are as follows:

Variety and locality, 1939		Mean difference A over B	
A	B	Mg. per gm.	Odds
Comet, Kennebunk, vs. Earliana, Kennebunk		+0.0417 ± 0.0054	630 to 1
Comet, Aroostook, vs. Earliana, Aroostook		+0.0346 ± 0.0048	499 to 1
Earliana, Aroostook, vs. Comet, Kennebunk		+0.0201 ± 0.0066	17 to 1

Analogous findings during 1938 showed the Comet variety to be vastly superior to the Penn State Earliana. Some of the mean differences may be set forth as follows:

Variety and locality, 1938		Mean difference A over B	Odds
A	B	*Mg. per gm.*	
Comet, Aroostook, vs. Earliana, Aroostook		+0.0325 ± 0.0053	158 to 1
Comet, Kennebunk, vs. Earliana, Kennebunk		+0.1422 ± 0.0113	1361 to 1
Comet, Aroostook, vs. Earliana, Kennebunk		+0.0043 ± 0.0044	2 to 1

The environment may exert an influence of such intensity as to cause a genetically high variety to produce less vitamin C than a genetically low variety. The third case shown in each of the two preceding summaries of mean differences reflects a trend typical of the reactions observed in this study. When 2 years' data from Aroostook and Kennebunk are analyzed together, the seasonal variations practically disappear, because Aroostook produced low- and Kennebunk produced high-vitamin-C tomatoes in 1938, and the opposite was true in 1939. The values were slightly more favorable in Kennebunk over the 2-year period, with a mean difference of 0.0373 ± 0.0221 mg. per gm. with odds of 6 to 1 in favor of Kennebunk.

Similar varieties from different localities show differences dependent upon the environmental conditions prevalent in each locality during the season. To illustrate this point, a few of the mean differences are set forth:

Variety and locality		Mean difference A over B	Odds
A	B	*Mg per gm.*	
1938			
Earliana, Aroostook, vs. Earliana, Kennebunk		+0.0564 ± 0.0007	Infinite
Comet, Aroostook, vs. Comet, Kennebunk		+0.1660 ± 0.0081	Infinite
1939			
Earliana, Kennebunk, vs. Earliana, Aroostook		+0.0618 ± 0.0108	168 to 1
Comet, Kennebunk, vs. Comet, Aroostook		+0.0547 ± 0.0088	240 to 1

Thus in comparing varieties within a rather limited locality, genes are found to exert a consistently significant influence. When the region is widened, e. g., into a State as large as Maine or even, as in this experiment, into areas delimited to less than one-half of the State, the influence of environment is manifested and apparently tends to neutralize the effect of genes, although this is purely a spurious phenomenon. In contrast, gene influence has an apparent neutralizing effect upon the environment. The genetic characteristic which effectively maintains high vitamin C constitutes a directive in the approach to the problem of increased vitamin C production for populations. Hence to ignore the importance of inherent qualities in respect to vitamin C would be an unwise omission. A variety nutritionally superior by virtue of its genes is proportionately superior even when subjected to an unfavorable environment.

The foregoing data give ample testimony to the doubtful nature of any one set of figures for the vitamin C content of a given fruit or vegetable. The necessity for using extreme care in evaluating diets and in assaying the available nutrients for specific crops or regions is manifest. It seems that observations covering several seasons' work on plants grown in widely separated localities would be more indicative of a truly genuine average value than would short-term experiments

of a few weeks' duration on localized samples. The results emphasize the importance of variety tests for more than one season on plants grown in the localities for which the varieties are intended.

The many known factors which play a part in the production of vitamin C during plant growth and in the retention of this food essential during storage, shipping, and cooking, as well as the less known influences which are gradually being brought to light, necessitate truly fine discrimination in utilizing the figures available in the literature. To achieve even a moderate degree of precision in the well-known types of vitamin C study and assay, it is apparent that an essential part of the experimental procedure would be direct analysis of the foods available in the region, whether locally grown, influenced as they are by seasonal factors, or of nonlocal origin, with consequent storage and shipping losses.

In view of the observations contained herein, it is clear that added to the influences imposed by maturity, variety, and metabolic rate, are those really large variations ascribable to seasonal influences.

The cause of these variations is uncertain. The writer hesitates to attribute the fluctuations in vitamin C values to the factors discussed above without more confirming evidence, but certain evidence indicates that preponderance of cloudy days, excessive rainfall, and low temperatures may well exert an unfavorable influence upon vitamin C synthesis.

In the experimental program outlined for this State, a slowness of approach is imposed by the very factors which come to light during the investigation of each unit. A survey of the vitamin C values of foods in so large a State requires adequate sampling within varieties as well as investigations along such lines as are discussed above. The ultimate aim of the program is to sift out varieties which are high enough in essential food factors to make fluctuations of this nature of less significance and to insure optimum intake to the consumer.

SUMMARY

Four varieties of tomatoes were grown in 1938 and 1939 and four varieties of cabbages were grown in 1939 in four widely separated localities in the State of Maine to determine the effect of geographical situation upon the production of vitamin C.

The tomato varieties chosen were Penn State Earliana, Bestal, Best of All, and Comet. In preliminary tests the first two were relatively low in ascorbic acid; the other two were at the upper end of the scale.

In 1938 it was observed that the tomatoes grown in Aroostook were lower in ascorbic acid content than those produced in the three more southern localities. The majority of the samples from Orono, Highmoor, and Kennebunk were over 0.06 mg. per gram greater in vitamin C content than those from Aroostook.

In 1939 the observations were continued with the experimental procedure unchanged. The tomatoes grown in Aroostook were lower in ascorbic acid than those grown in Orono but higher than those grown at Highmoor and Kennebunk. The majority of the samples were more than 0.04 mg. per gram higher in ascorbic acid content in Aroostook, Orono, and Highmoor than in Kennebunk.

An analogous experiment in which four varieties of cabbages were used for the test crop confirmed the 1939 evidence obtained from tomatoes.

The above results demonstrated that environmental agencies markedly influence the synthesis of vitamin C in tomatoes and cabbages, and that geographical situation is not a contributing factor except insofar as environmental conditions are consistently characteristic of that situation.

An analysis of available weather data provided a certain basis for the assumption that sunlight, rainfall, and probably temperature may all be causal agents in the variations in ascorbic acid.

Paralleling maturation of the tissue, a definite rise in ascorbic acid concentration in the tomato and a decline in the cabbage was observed. These phenomena were related to geographical situation to the extent that maturity rate was hastened or delayed by the climatic conditions prevailing throughout the growing season in any one region.

Although variations effected by environment may be of greater magnitude than varietal differences, this does not lessen the importance of the varietal values. It is obvious that if a variety of low ascorbic acid content is subjected to adverse environmental influences, the tomato may well be rendered useless as a source of vitamin C. On the other hand, a high vitamin variety subjected to the same unfavorable conditions could still contribute materially to vitamin C requirements.

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HEIGHT OF CORN AS A FACTOR IN EGG LAYING BY THE EUROPEAN CORN BORER MOTH IN THE ONE-GENERATION AREA [1]

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INTRODUCTION

Every investigator who has had occasion to record the number of egg masses of the European corn borer (*Pyrausta nubilalis* (Hbn.)) laid on plots of corn (*Zea mays* L.) of varying height within experimental fields has noted that where one generation occurs the tall corn receives more eggs than the short corn. The economic significance of this fact lies in its relation to the recommendation that corn be planted late as a control measure against the corn borer. Corn in fields planted late obviously would have shorter stalks at the time the eggs are laid than corn in fields planted early, and might show the same lower attractiveness to the ovipositing moths that has been noted in the late-planted corn in planting-date plot experiments. Since the comparative number of eggs deposited is one factor to be considered in the formulation of a reliable recommendation respecting the best time to plant corn to escape borer damage, it was deemed worth while to study the comparative numbers of egg masses laid in fields of different corn heights over county-wide areas.

Available for this study were the data [3] obtained over the 4-year period 1930–33 from 1,600 fields of corn distributed in several States in the one-generation area of the European corn borer. The original field records on file at the Federal laboratory for European corn borer research at Toledo, Ohio, were used.

SURVEY METHODS

The four annual surveys made by the staff of the laboratory during this period to ascertain the abundance of the egg masses of the borer covered 36 counties or districts of Michigan, Ohio, and New York. Although the data obtained from one field afforded the primary sampling unit, two larger groups were used in the analyses. Ten fields constituted what is here called a unit area, and three unit areas covering the entire county or group of two counties comprised a

county area. The data included in this analysis came from 159 unit areas within 53 county areas and 1 additional unit area. Six of these unit areas were surveyed in 1933 and the others in 1930, 1931, and 1932.

The same general procedure was followed each year. The survey for each county area was made by one man. He was given a map upon which 30 points were indicated by dots. In the placing of these dots each unit area had been divided into 10 nearly equal parts and a point selected along a road as near as possible to the center of each part. When approaching the point indicated on the map, the man selected the first cornfield encountered. Every third day over a period of 18 to 21 days he made counts of the egg masses on five widely separated plants from each of six sections of the field. By counting only fresh, white egg masses he was reasonably certain that they had been laid since the preceding count, as older masses are of a darker color owing to the development of the embryos within the eggs.

The average height of the corn was recorded each time a field was visited, by measuring the height of 10 single plants taken at random if the corn was drilled, or of the plants in 10 hills if the corn was planted in hills. In either case the measurement was from the ground to the tip of the longest portion of growth when the leaves were gathered in the arms and held upward.

ASSOCIATION OF NUMBER OF EGG MASSES WITH HEIGHT OF CORN AMONG THE COUNTY AREAS

In the first analysis of the data the 53 county areas were divided into 3 groups of 13 and 1 group of 14 on the basis of the average height of corn in the area. The areas having the shortest, below-average, above-average, and tallest corn plants, respectively, provided the data for these 4 groups. Within these groups another grouping of the fields was made, depending again on the height of the corn. As each county area included 30 fields, a group was made for each area of the 10 fields with the shortest corn plants. Lastly, the data were arbitrarily divided into those obtained during the first half and those obtained during the last half of the oviposition period. For these final subgroups the mean corn heights and the mean numbers of egg masses per 100 plants were calculated. The results are given in table 1.

It may be noted in table 1 that about twice as many egg masses were found in the county areas with corn above average in height as in the areas with below-average corn. If the higher levels of egg masses were the result of greater numbers of moths, were the moths attracted to the areas of thriftier, taller corn from areas of shorter corn, or did they survive from higher levels of fall populations of borers that tended to accumulate in areas of tall corn? Regarding the latter possibility data show that higher levels of egg masses in areas of tall corn were not preceded by higher levels of fall populations of borers. Supplementary data from the 27 county areas of above-average corn show that the populations of borers in the fall preceding the infestations of egg masses averaged 29.1 per 100 plants as compared with 42.6 from the 26 counties of below-average corn.

For a study of the factors associated with higher levels of fall populations of borers in areas of shorter corn, the data from certain counties grouped according to locality were available. The data taken in 1931

TABLE 1.—*Number of egg masses of the European corn borer on corn in the fields with shortest corn grouped according to county areas in Michigan, Ohio, and New York, 1930–33*

Corn-height group	County areas in group	Part of oviposition period	Mean date of record	Total egg masses per 300 plants	Mean corn height in county areas	Ten fields in county area with shortest corn	
						Mean egg masses per 100 plants	Mean corn height
	Number			*Number*	*Inches*	*Number*	*Percent of total*	*Inches*
Shortest	13	First half	July 7	41.1	30.33	4.8	11.7	23 0
		Last half	July 16	34 7	43.07	6.1	17.6	34.4
		Mean or total		75.8	36.70	10.9	14 4	28.7
Below average	13	First half	July 7	42 6	34.87	6 4	15 0	26.9
		Last half	July 16	31.8	50.57	6 0	18 9	40 4
		Mean or total		74.4	42.72	12 4	16.7	33.6
Above average	13	First half	July 5	118.4	41 83	25 1	21 2	34.1
		Last half	July 15	55.7	60 17	15 1	2/.1	50 2
		Mean or total		174.1	51 00	40 2	23 1	42 2
Tallest	14	First half	July 4	102.4	49 00	25 4	24.8	41.9
		Last half	July 13	44 0	70 93	13 7	31 1	62 3
		Mean or total		146.4	59 97	39 1	26.7	52 1

and 1932 were used, because 18 of the same counties were surveyed both years, whereas only 8 of the same counties were surveyed in all 3 years. The counties surveyed may be considered in three groups on the basis of height of corn. The tallest corn was produced in a block of counties in northwestern Ohio. Corn intermediate in height was grown in counties bordering Lake Erie or separated from it by not more than 1 county in northwestern Ohio or southeastern Michigan and located north and east of the counties in the first group. The shortest corn was grown in New York State in counties bordering Lake Ontario or Lake Erie. The counties in each group are given in table 2 together with the mean corn heights, the mean numbers of egg masses per 100 plants, and the fall populations of borers. To assist in interpretation of the data the numbers of eggs per mass, the numbers of

TABLE 2.—*Mean heights of field corn, mean numbers of egg masses per 100 plants, and related data, in grouped counties differing in corn height, Michigan, Ohio, and New York, 1930–32*

Counties as grouped	Year	Mean height of corn	Egg masses per 100 plants	Eggs per mass	Eggs per 100 plants	Borers surviving	Borers per 100 plants
		Inches	*Number*	*Number*	*Number*	*Percent*	*Number*
Tall corn--Williams, Defiance, Paulding, Van Wert, Fulton, Henry, Wood; all in Ohio	1930	....	....	....	....	....	9.0
	1931	66.4	33.2	12.2	405	6.5	26.3
	1932	60.5	50.3	22.5	1,132	3.2	35.8
	Average	63.5	41.8	17.4	....	4.9	[1] 31.1
Intermediate corn—Lenawee and Monroe, Mich.; Lucas, Ottawa, Sandusky, Erie, and Huron, Ohio	1930	....	....	....	....	....	20.7
	1931	55.7	33.3	14.5	484	9.4	45.3
	1932	51.9	152.1	23.1	3,518	1.4	49.0
	Average	53.8	92.7	18.8	....	5.4	[1] 47.2
Short corn — Chautauqua, Jefferson, Wayne, and Monroe; all in New York	1930	....	....	....	....	....	24.0
	1931	39.5	31.6	15.1	477	23.3	111.5
	1932	42.7	21.7	27.5	598	13.8	82.6
	Average	41.1	26.7	21.3	....	18.6	[1] 97.1

[1] Average for 1931 and 1932 only.

eggs per 100 plants, and the percentages of borers surviving from the numbers of eggs laid are included.

Averages of 22 and 15 fields per county were surveyed in June and July of 1931 and 1932, respectively, to determine the number of egg masses per plant sample. The average number of eggs per mass was determined on the basis of samples of 100 masses taken, in lots of 25, 4 times during the season from each country or area where 30 fields were surveyed. In the fall 20 or 25 fields in each county were surveyed to determine the fall populations of borers, but no attempt was made to use the same fields that were used for the earlier counts.

In 1931 the shortest corn averaged 26.9 and 16.2 inches shorter than the tallest and intermediate corn, respectively, but the population of borers in corresponding counties the preceding fall was 166.7 and 15.9 percent more dense. In 1932 the shortest corn averaged 17.8 and 9.2 inches shorter than the tallest and intermediate corn, respectively, and the population of borers the preceding fall was 324.0 and 146.1 percent more dense. Although data are not available to compare the heights of corn in 1933 with the fall populations of borers in 1932, nevertheless the fall population of borers in the shortest corn in 1932 was 130.7 and 68.6 percent more dense than in the tallest and intermediate corn, respectively. The higher levels of fall population of borers in the shortest corn in 1931 and 1932, as may be noted from table 2, were the result of more eggs per mass and a much higher borer survival in the areas of shortest corn.

It is conceivable that the number of egg masses laid in an area might be determined by the size of the mass. If conditions caused the laying of smaller masses in one area than in another, more masses might be laid in that area provided the complement of eggs of the moths was not less. The difference between 27.5 and 22.5, the numbers of eggs per mass in 1932 in the areas of shortest and tallest corn (table 2), is not great enough to account for the larger number of egg masses in the areas of tallest corn. It appears possible that in the areas of shortest corn less favorable conditions prevailed during the pupation period, as well as during the dormant period of the borers, and reduced the fecundity of the females. Weather during moth flight is also believed to determine the percentage of the complement of eggs that are laid.

Houser and Huber [4] state:

Since moths are attracted more strongly to the best corn it would appear logical to assume that if the fields of good corn were widely separated the moths would necessarily fly greater distances to satisfy their reproductive urge, for the fulfillment of this urge is imperative for the success of the species.

While the author does not hold the view that moths will fly great distances to seek good corn, it appears that fields within and near the border of an area of tall corn would undoubtedly attract some moths from an adjacent area of less thrifty corn. How far moths from outside would be attracted to fields within an area is not known. One or more of the several factors considered in the foregoing discussion might have contributed toward a higher level of egg masses in northwestern Ohio and southeastern Michigan, where the corn was relatively tall during moth flight, than in the counties bordering Lake Ontario in New York, where the corn was short.

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ASSOCIATION OF NUMBER OF EGG MASSES WITH HEIGHT OF CORN AMONG FIELDS WITHIN THE UNIT AREAS

PRELIMINARY ANALYSES

The preceding data show that factors other than height of corn probably caused greater numbers of egg masses to be laid in groups of counties with tall corn as compared with groups of counties with shorter corn. The relationship between the number of egg masses and the height of corn was studied also on the basis of field data taken in most cases from areas covering only a third of a county. Before this study was made five preliminary analyses were necessary.

(1) The first of these analyses eliminated from further consideration the data from 40 of the 160 unit areas available. The 40 unit areas having less than 5.1 egg masses per 30 plants were not used, because many of the fields were not infested. In unit areas where the infestation averaged less than 1 egg mass per 30 plants, only 32.5 percent of the fields were found to be infested; where the density of infestation averaged from 1 to 1.9 masses per 30 plants, 60 percent of the fields were infested; and where the infestation averaged from 4 to 5.9 masses per 30 plants, 93.5 percent of the fields were infested.

(2) It was determined that the data were homogeneous with respect to the distribution of the heights of the corn plants among the fields within the areas. This was accomplished by obtaining the difference between the height of corn in the tallest and shortest fields within each unit area. The absence of a significant change in this difference was indicated when the regression of the differences on the mean heights of corn in the unit areas was calculated. The coefficient of regression (b) was found to be $+0.005 \pm 0.0656$ inch. When the data were classified according to years, it was found that for 1930, 1931, and 1932 the difference in heights averaged 24.0, 21.5, and 26.0 inches, respectively. Since these averages differ only slightly from the 24-inch average for the data of all unit areas, and since the regression coefficient did not differ significantly from zero, it appears that during these years there was no marked tendency for a narrowing of the range between the heights of the corn in the field of tallest corn and in the field of shortest corn such as might be expected in years when the weather necessitated the planting of corn within a short period late in the season. In this respect, then, the data from the 120 unit areas may be regarded as one homogeneous set.

(3) It was determined that the regression of the number of egg masses per 30 corn plants on the height of corn for fields within the unit areas was on an average proportional to the mean number of masses found in the 10 fields examined in each area. The proportional relationship would necessarily occur in the absence of disturbing factors because of the mathematical considerations. An average increase of 1 egg mass per 30 plants from field to field for each increase of an inch in the height of the corn becomes an increase of 2 masses under conditions of infestation with twice the former number of egg masses in each field. This relation is purely mathematical. Increasing proportionally the magnitude of each item of the dependent variable while each item of the independent variable remains the same causes a proportional increase in the regression coefficient. This phase of the analysis was continued to determine if disturbing factors were involved.

Accordingly, the regression (*b*) of the number of egg masses per 30 plants in the individual fields (*Y*) on the height of corn in the fields (*X*) was calculated for each unit area. If it is assumed that the regression coefficients obtained were in fact proportional to the mean number of egg masses in each area, and each regression coefficient is then expressed as a percentage of the mean number of masses, the effect of egg-mass density may be disassociated from the effect of other possible factors on the regression coefficients. As subsequent data show, the mean level of corn height in the unit areas was the only other factor associated with the comparative regression coefficients. The comparative regression coefficients were therefore corrected for the effect of their regression on the mean level of corn height and converted back to *b* values not expressed as percentages of the mean number of masses.
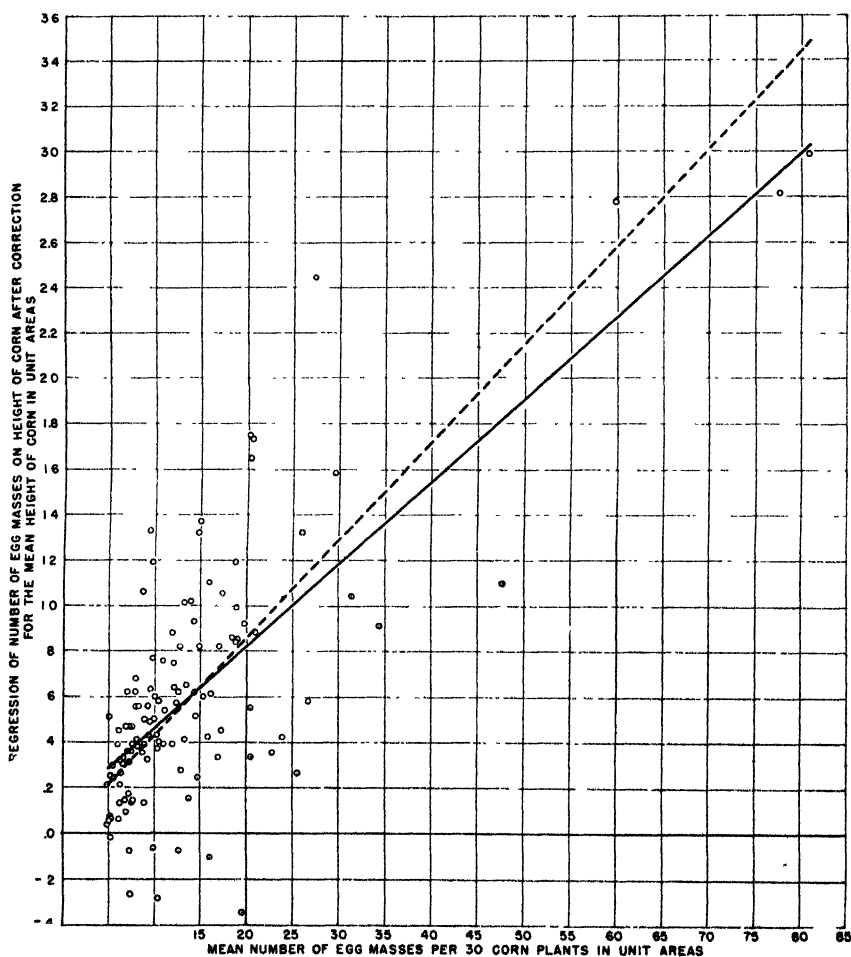
FIGURE 1.—Coefficient of regression of *Y* on *X* within unit areas plotted against the mean level of egg masses after correction of the coefficient for the mean height of corn. The unbroken line is the regression line as calculated; the broken line is the regression line expected on the basis of proportionality between the regression coefficients and the mean level of egg masses.

In figure 1 the corrected *b* values are plotted against the mean numbers of egg masses in the unit areas. The unbroken regression line was fitted to the plotted points by the method of least squares.

The next step was to compare the regression of the corrected *b* value on the mean level of egg masses with the regression expected on the basis of a proportional relationship between the two factors. The corrected *b* values were found to increase on an average 0.0362 ± 0.00296 for each increase of 1 egg mass per 30 plants. The mean number of masses per 30 plants in the 120 unit areas was 14.23. The mean of the corrected *b* values was calculated to be $+0.612 \pm 0.0350$. Exact proportionality would yield a straight line passing through the origin and the general mean. Its slope would then be 0.0430 ± 0.00246. The broken line in figure 1 shows this expected increase. The difference between this expected increase and the actual increase is 0.0068 ± 0.00385, giving a *t* value of 1.766, which is somewhat less than 1.980, the value required for significance. It appears that the corrected *b* values show a closely proportional relationship to the mean level of egg masses in the unit areas.

An inspection of figure 1 suggests that the meagerness of the data between levels of 35 and 82 egg masses might have had an undue effect on the position of the regression lines. Accordingly, the data were recalculated with the exclusion of the data from the 4 unit areas between those levels. The corrected *b* values increased on an average 0.0387 ± 0.00559 for each increase of 1 egg mass per 30 plants as compared with an expected increase of 0.0442 ± 0.00284 on the basis of proportionality. The difference of 0.0055 ± 0.00627 is not significant. The exclusion of the 4 unit areas strengthens the previous conclusion.

(4) It was determined that no other factors were involved in the association of the dependent with the independent factor shown in figure 1. The effect of the mean level of corn height has been accounted for in the construction of figure 1. Other possible factors are location of the unit areas and the year in which the data were obtained. The variability among the 120 corrected regression coefficients was compared with the error of sampling within the unit areas, after the variability among the coefficients due to the proportional relationship with the mean level of egg masses had been taken out. The standard error of the coefficients around the unbroken regression line in figure 1 is 0.3831. The variance of the regression of Y on X was calculated for each unit area, and from the mean variance the generalized standard error of *b* was found to be 0.4117. The near equality of these two values led to the conclusion that, within the several States of the one-generation area, including the unit areas, and within the period covered, factors other than the general level of corn height for which the regression coefficients were corrected had no effect on the regression of Y on X.

(5) A study was made to determine whether the relation between number of egg masses per 30 corn plants and the height of corn among fields within the unit areas was linear or curvilinear. Because of the extreme variability of the data, a grouping of the unit areas was necessary to stabilize the data sufficiently to make the study. The corn-height data were arranged according to the average height of corn for each of the 120 unit areas. Different height levels were then obtained by dividing the unit areas into 20 equal groups. Then the fields in each unit area were grouped according to their deviation

from the mean height. Four classes were made with intervals of 4.0 inches, and 2 other classes included any fields deviating either positively or negatively by more than 8.0 inches from the mean height. The number of egg masses per 30 plants in each field was expressed as a percentage of the mean number in the 10 fields of each area. Then the corn-height deviations of the fields included within each of the 6 height classes for each of the major groupings of the unit areas were averaged. The corresponding number of egg masses on a percentage basis were also averaged. A visual examination of the plotted data indicated a linear regression of number of egg masses on corn height. It appears, therefore, that because the relation between the two variables was linear within areas as few as 6 unit areas, it is reasonably safe to conclude that such would have been the case within individual unit areas if the sampling had been more intensive within the areas.

REGRESSION OF NUMBER OF EGG MASSES ON HEIGHT OF CORN

The information gained from the preceding analyses assures that the study of the regression of the number of egg masses per 30 plants on the height of corn from field to field within the unit areas may proceed on a linear basis with all other factors accounted for, when the regression coefficients of Y on X are converted to percentages of the mean numbers of egg masses in the unit areas. An average difference of 24 inches between the tallest and shortest corn within the unit areas was found regardless of the mean level of corn height.

The comparative regression coefficients are plotted in figure 2
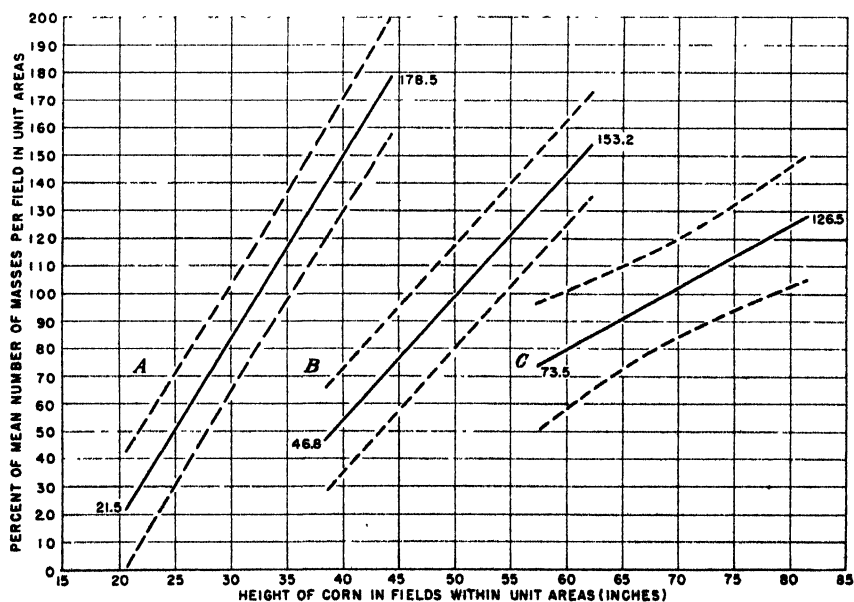
FIGURE 2.—Coefficient of regression of Y on X within unit areas, expressed as a percentage of the mean of Y, plotted against the mean height of corn. The broken-line curves give the limits (for odds of 19 to 1) enclosing the true regression line whose estimate is the unbroken line.

against the mean height of the corn in the fields within the unit areas. For each increase of an inch in the general level of corn height the coefficient of regression (*b*) of *Y* on *X*, expressed as a percentage of the mean of *Y*, was found to decrease 0.117 ± 0.0277. With 118 degrees of freedom the regression may be regarded as highly significant. The standard error of the plotted points around the regression line was found to be 2.931 percent as compared with a within-unit-area error of sampling for *b* of 2.810 percent. The mean of the dependent variable is 4.43 ± 0.072 percent, and that of the independent variable is 50.4 inches.

The next step is to use the information given by the data in connection with figure 2 to show the average conditions encountered within unit areas having specified levels of corn height. Mean heights of 32.4, 50.4, and 69.4 inches were selected. At these levels the best estimates of the regression of *Y* on *X* are calculated to be 6.54 ± 0.566, 4.43 ± 0.268, and 2.21 ± 0.526 percent, respectively. Within unit areas where the corn averaged about 32.4 inches in height, an average of $100 - 12(6.54)$, or 21.5 percent of the mean number of egg masses, was counted in the fields with shortest corn as compared with $100 + 12(6.54)$, or 178.5 percent of the mean number of egg masses, in the fields with tallest corn. In these areas on an average 12.0 percent as many masses were counted on the shortest corn as on the tallest corn. Similarly, within unit areas averaging about 50.4 inches in corn height an average of 46.8 percent of the mean number of egg masses was counted in the fields with shortest corn as compared with 153.2 percent in the fields with tallest corn, or 30.5 percent as many in the fields with shortest corn. Within unit areas averaging about 69.4 inches in height an average of 73.5 percent of the mean number of egg masses was counted in the fields with shortest corn as compared with 126.5 percent in the fields with tallest corn, or 58.1 percent as many in the fields with shortest corn.

The foregoing percentages of the mean number of egg masses are plotted in figure 3 against the height of the corn in the fields of shortest and tallest corn, respectively, within a unit area and are connected by straight unbroken lines. The vertical scale represents numbers of egg masses as percentages of the mean number of masses in all fields within a unit area. Hence the mean number of masses per field is 100 percent on the scale. The midpoint of each regression line coincides with the point determined by plotting 100 percent against the mean height of corn. The length of the lines covers a range extending from 12 inches below to 12 inches above the mean height. The range of 24 inches in height was the average difference between the field of shortest corn and the field of tallest corn within a unit area.

The general level of corn height within a unit area has been shown to have a highly significant effect on the uniformity of the distribution of the egg masses among the fields. When the corn height averaged 32.4 inches, only 12.0 percent as many masses were counted on the shortest corn as on the tallest corn, as compared with 58.1 percent as many when the corn averaged 69.4 inches high. This difference may be attributed to some factor associated with the difference of 37 inches in the general level of corn height. This factor might be explained as a decreasing difference in the attractiveness to the corn borer moth between fields of tall corn and fields of short corn as the general level of the height of corn in the fields within an area increased.

This explanation is in harmony with the increased attractiveness of the fields of shortest corn during the last half of the oviposition period. The data given in table 1 show that 23.7 percent of the masses laid during the last half of the oviposition period were laid in the third of the fields with the shortest corn as compared with 18.2
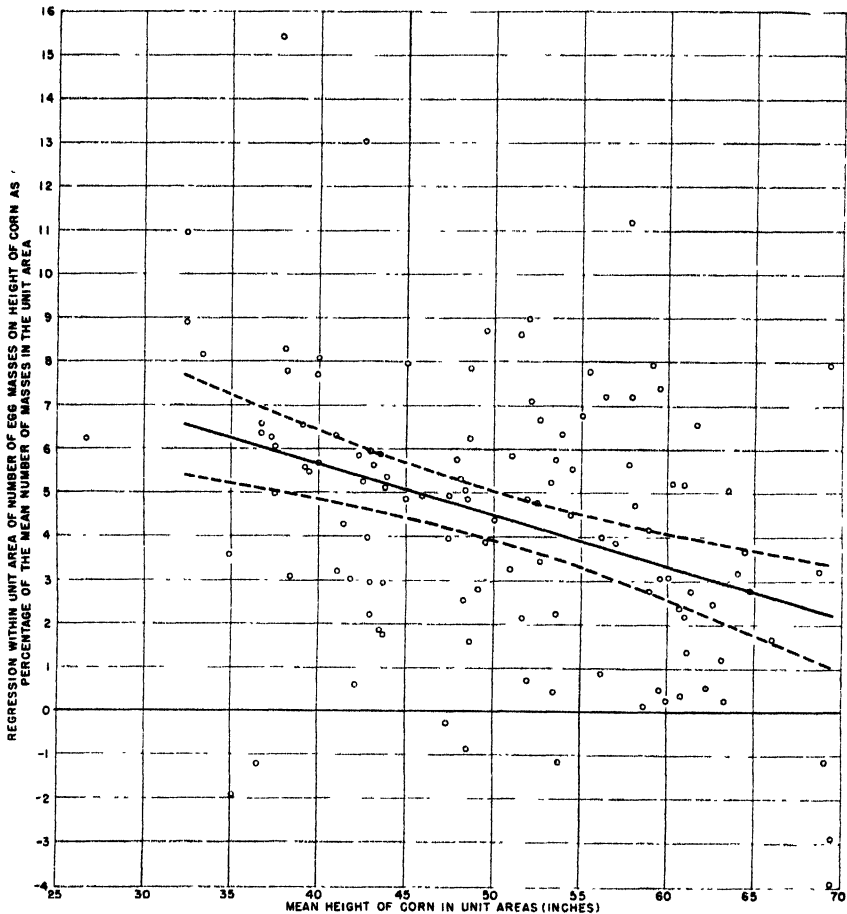
FIGURE 3.—Percentages of the mean number of egg masses of the European corn borer per cornfield in unit areas that were laid in single fields of different corn height, when the mean height of the corn in all fields in the unit areas approximated (A) 32.4, (B) 50.4, and (C) 69.4 inches. The broken curves give the limits enclosing the true regression line (for odds of 19 to 1) when the egg masses on 30 plants in each of 36 fields taken at random within the unit areas are counted and the level of egg density ranges from 10 to 22 egg masses per 30 plants.

percent laid in the same fields during the first half of the period. That is, there was a partial shift in egg laying away from the fields of taller corn toward the fields of shorter corn as the season advanced and the general level of corn height increased. On the basis of the total number of egg masses laid during the oviposition period, and in harmony with this phenomenon, the fields of shorter corn appear to

have attracted proportionately more moths when the general level of corn height was high than when the general level was low. Accordingly, the distribution of the egg masses among the fields was more uniform within areas of tall corn.

The attractiveness to the corn borer moth of corn in late-planted fields as compared with corn in fields planted earlier is one of the four major factors [5] to be considered in recommendations for the best time to plant corn to escape maximum damage by the borer. Although the planting dates for the fields surveyed in the present study were not determined, it is obvious that the earliest planted fields within an area would be in the group with tallest corn at the time of moth flight, whereas the latest planted fields would be among the fields in the group with shortest corn. The fields of shorter corn received, in general, smaller numbers of egg masses within 112 of the 120 unit areas studied. Hence late-planted fields had some advantage over early-planted fields from this factor alone. If the conditions giving the results obtained from 1930 to 1933 may be considered as typical, the advantage of late-planted fields would be greatest in the localities and years when the level of corn height is comparatively low at the time of moth flight. In areas where the corn was short at the time of moth flight, 8.3 times as many egg masses were laid in the fields of tallest corn as in the fields of shortest corn, whereas in areas where the corn was 37 inches taller at the time of moth flight only 1.7 times as many masses were laid in the fields of tallest corn as in the fields of shortest corn. The advantage of the latest over the earliest planted fields in this case was only 20.5 percent as great in the areas with a high level of corn height as in the areas with a low level of height.

ASSOCIATION OF NUMBER OF EGG MASSES WITH HEIGHT OF CORN AMONG PLOTS WITHIN FIELDS

Consistent decreases in the number of eggs laid with decreases in the height of corn among plots occurred in experiments carried on by Neiswander and Huber.[6] In the present study plot experiments conducted by the Illinois Natural History Survey and the Illinois Agricultural Experiment Station from 1929 to 1934 were available for determining the association of number of egg masses with height of corn among plots within a field. Each plot was 9 hills in size during 5 seasons and 12 hills during 1 season. The number of plots in the field during the period ranged from 64 to 132. The height of the corn in the plots, measured from the ground to the tip of the longest portion of growth of the hill when the leaves were held upward, varied because of the strain of corn, the date of planting, and the location of the plot in the field. The number of egg masses laid in each plot was found by examining all plants at least once every 5 days and identifying them with clips so that the same masses would not be counted twice.

The data were considered as one set of data irrespective of strain or other factors. The mean number of egg masses (Y) was found to bear a linear relationship to the height of corn (X). The coefficients of regression of Y on X were calculated and adjusted to express the change in the number of egg masses per 30 plants for each change of

[5] The other factors are being considered in supplementary studies.
[6] NEISWANDER, C. R., and HUBER, L. L. HEIGHT AND SILKING AS FACTORS INFLUENCING EUROPEAN CORN BORER POPULATION. Ent. Soc. Amer. Ann. 22 527-542, illus. 1929.

1 inch in the height of corn. The adjusted values were then expressed as percentages of the respective mean number of egg masses per 30 plants in all plots. A comparison between the plot data within fields and the field-to-field data, taken from figure 2 for equivalent heights of corn, is given in table 3.

TABLE 3.— *Regression of number of egg masses of the European corn borer per 30 plants of corn, expressed as a percentage of the mean number of masses, on the height of corn among plots within fields, compared with the regression among fields, near Toledo, Ohio, 1929-34*

Year	Mean date of oviposition	Among plots within fields			Among fields (readings taken from the unbroken line in fig. 2)	
		Estimated mean height of corn (X) on mean date of oviposition	Mean number of egg masses per 30 plants (Y)	Regression of Y on X	Mean height of corn (X) during oviposition period	Regression of Y on X
		Inches	*Number*	*Percent*	*Inches*	*Percent*
1929	July 8	38.8	11.6	6 44±0.506	38 8	5.79
1930	July 4	40.0	24.2	5.05±0.449	40.0	5.65
1932	July 2	52 2	14 1	3.07±0.203	52.2	4.22
1931	July 5	59.5	4.7	4.31±0.576	59.5	3.37
1933	July 4	59.6	16.4	2.61±0.597	59.6	3 35
1934	July 2	62.5	8.6	2.88±0.283	62.5	3.01
Mean	July 4	52.1	13.3	4 06±0 188	52 1	4.23

As shown in table 3, for each increase of 1 inch in corn height from plot to plot within fields there was an average increase of 4.06 ± 0.188 percent, or 0.54 egg mass, in the mean number of egg masses per 30 plants. An average increase of 4.23 percent is shown between fields having average equivalent heights of corn. Hence it is concluded that moths of the corn borer are attracted as much more to fields of tall corn than to fields of short corn as they are to plots of tall corn than to plots of short corn within fields.

SUMMARY

In the one-generation area of the European corn borer (*Pyrausta nubilalis* (Hbn.)) investigators have found that plots of tall corn always contain more egg masses than plots of short corn. Because of the relation of height of corn to time of planting, this fact is important when control measures are being considered. A study has therefore been made of the distribution of egg masses among fields of corn over county-wide areas.

The egg masses were counted on 30 corn plants in each of 10 corn-fields taken at random in each of 160 unit areas in Michigan, Ohio, and New York from 1930 to 1933, inclusive. A unit area was usually a third of a county or a group of two counties. The average plant height during the oviposition period was determined for each field.

Data from unit areas within which all the fields examined were infested showed that factors such as location of the unit areas and year in which the data were obtained did not affect the distribution of egg masses among the fields within the unit areas. The mean level of corn height, however, was found to have a marked effect on the distribution of egg masses among the fields.

More egg masses were laid in the fields of tall corn within an area than in the fields of short corn, but the difference was less marked in

areas where the general level of corn height was high.   When the corn within unit areas averaged 32.4 inches in height, 8.3 times as many egg masses were laid in the fields of tallest corn as in the fields of shortest corn, whereas in unit areas where the corn averaged 69.4 inches at the time of moth flight only 1.7 times as many egg masses were counted in the fields of tallest corn.

When the data were grouped on the basis of counties without regard to the unit areas within them, about twice as many egg masses were found in the counties with corn above average in height as in the counties with below-average corn.   This condition did not seem to result from higher levels of early summer infestations of egg masses in areas of tall corn preceded by higher levels of fall populations of borers. Other factors are discussed.

Moths were found to be attracted as much more to fields of tall corn than to fields of short corn as they were to plots of tall corn than to plots of short corn within experimental fields.

COMPARATIVE RIPENING OF BEEF FROM GRASS-FATTENED AND GRAIN-FATTENED STEERS [1]

By Nicholas G. Barbella, *associate biochemist*, Bernard Tannor, *junior chemist*, and O. G. Hankins, *senior animal husbandman, Bureau of Animal Industry, United States Department of Agriculture*, and R. E. Hunt, *animal husbandman, Virginia Agricultural Experiment Station*

INTRODUCTION

Cattle fed and finished on grass usually bring a lower price on United States markets than similar cattle finished on grain. The reasons advanced for the price discrimination are many. One of the principal objections raised is that the beef from cattle fattened on grass does not keep or ripen satisfactorily. In order to obtain much-needed information on the "grass beef" problem, a cooperative study was made by the Virginia Agricultural Experiment Station, the Virginia State Division of Markets, and the United States Department of Agriculture Bureau of Animal Industry and Agricultural Marketing Service. The first experiment was conducted in 1937–38 and was followed by similar experiments in 1938–39 and 1939–40. The present paper reports the phase of this study concerned with the comparative ripening qualities of the two kinds of beef.

METHODS OF EXPERIMENTATION

Beef-type steers produced in southwestern Virginia and averaging low Good in grade of feeder were selected for this study. In each of the 3 experiments, 40 steers about 2½ years old and of similar breeding, weight, and condition were used. All cattle were wintered in 1 group on a medium plane of nutrition, and they lost an average weight of approximately 100 pounds.

At the beginning of the grazing season the cattle were divided into 2 comparable groups of 20 head each. Group 1 was turned on blue-grass (*Poa pratensis*) pasture and received only grass and block salt for approximately 5 months. Group 2 was placed in dry lot and fed shelled corn, cottonseed meal, and mixed hay. These cattle had access to block salt and were fed to the same degree of fatness as those on grass. The feed required consisted of 649 pounds of corn, 80 pounds of cottonseed meal, and 412 pounds of hay per 100 pounds of grain. At the end of the feeding period each group was divided as equally as possible into two comparable subgroups of 10 animals each. One subgroup in each instance was shipped to the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., and the other to the Jersey City market.

At Beltsville the cattle were slaughtered and the carcasses were chilled to approximately 34° F. Ether extract of the eye muscle of the ninth-tenth-eleventh-rib cut was determined for each of the 20 carcasses. Three pairs of these carcasses were selected for the study reported in this paper. Each pair consisted of a carcass of a grass-fed steer and that of a grain-fed steer. The pair mates were of as nearly the same fat content of eye muscle as was possible to obtain from the 10 carcasses representing each type of feeding. From these 3 pairs of carcasses the 12 sixth-seventh-eighth-rib cuts were used for the study on ripening.

Beginning 15.7 days after slaughter, on the average, in the 3 experiments, the analytical work on the rib samples from the right side of the 6 carcasses was begun. The eye muscle was removed from the sixth-rib cut, ground 3 times, and mixed thoroughly for chemical analysis. Preparatory to determination of the expressible-juice content and to tests for flavor and aroma, the seventh-eighth-rib cut was cooked at an oven temperature of 125° C. to an internal meat temperature of 58° C. The juice content was determined by a method, developed by the Bureau of Animal Industry, which involves the testing of small samples of cooked meat in a hydraulic press under certain conditions of temperature, pressure, and time.[2] Flavor and aroma were judged by a committee of qualified persons.

To subject the meat to an extremely critical test, the sixth-seventh-eighth-rib cuts from the left side of the carcass were stored at 33° to 36° F. for 50 days. At the end of this period, all moldy and slimy surfaces were removed from the samples. The same procedure was then followed as described for the 15.7-day period.

To measure the autolysis, or break-down, of muscle tissue during ripening, the content of amino and nonprotein nitrogen, as well as that of other tissue components, was determined. Moisture, ether extract, total nitrogen, amino nitrogen (by formol titration), and ash determinations were made according to methods recommended by the Association of Official Agricultural Chemists.[3] Nonprotein nitrogen was determined by treating 5 gm. of ground meat with 100 cc. of 10-percent trichloroacetic acid. Sulfydryl determinations were made according to the Okuda iodate method[4] with the use of Lavine's modification.[5] Reducing substances were determined by the Somogyi-Shaffer-Hartmann method, as given in Hawk and Bergeim.[6] Although reducing values were obtained by this method, the values are expressed as total reducing substances in terms of glucose. The reduction obtained is probably due to intermediate products formed during the conversion of glycogen. The data obtained on total nitrogen, amino nitrogen, nonprotein nitrogen, and sulfydryl were analyzed statistically by the use of Fisher's *t* test.[7]

[2] The method used for determining expressible juice was that briefly given in the Reports of the Chief of the Bureau of Animal Industry for 1937 and 1939, and later improved. A paper giving the details of the method has been prepared for publication.
[3] ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . Ed. 4, 710 pp., illus. Washington, D. C. 1935.
[4] OKUDA, YUZURU. A NEW METHOD FOR THE DETERMINATION OF CYSTINE IN PROTEINS (THE IODINE METHOD). Jour. Biochem. 5: 217–227. 1925.
[5] Personal communication from T. F. Lavine, Lankenau Hospital Research Institute, Philadelphia, Pa.
[6] HAWK, PHILIP B., and BERGEIM, OLAF. PRACTICAL PHYSIOLOGICAL CHEMISTRY. Ed. 11, 968 pp., illus. Philadelphia. 1937.
[7] FISHER, R. A. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 pp., illus. Edinburgh and London. 1930.

EXPERIMENTAL RESULTS

The paired samples of the ninth-tenth-eleventh-rib cuts used in the 3-year study were of approximately equal fatness, as indicated by the ether-extract content of the eye muscle. The differences in the ether extract among the nine pairs at time of slaughter ranged from 0 to 0.09 percent, the mean difference being 0.051 percent. Table 1 presents data on the comparative composition of the beef after ripening for an average period of 15.7 days. These data show that the beef from the grain-fed and grass-fed steers differed little in moisture, ash, protein, and ether extract. Any changes in these constituents that occurred during the short period of ripening were similar for the two types of beef. In the amino-nitrogen determinations, there was no difference, on the average, between the meats representing the two types of fattening. For each of the 3 years, as well as for the average of the 3 years, there were only small differences in nonprotein nitrogen. The total reducing substances show no appreciable difference, but the percentage of sulfydryl in the beef from the grass-fed cattle was appreciably higher than that in the grain-fed cattle. However, the higher sulfydryl content was not interpreted as indicating that more ripening had occurred in the beef from the grass-fed cattle.

Table 1 also presents data on the 50-day ripening period for the same constituents. As was found after the short period of ripening, the differences between the two kinds of beef in moisture, ash, protein, and ether extract were of minor importance. Furthermore, such small changes as did occur in these constituents as a result of the longer ripening period were similar in the beef from the grain-fattened and grass-fattened cattle.

On the other hand, the values for nonprotein nitrogen and amino nitrogen were considerably higher after 50 days of ripening than after an average of 15.7 days. The increase represents what normally occurs in ripening meat as a result of attack by enzymes and bears out the results of previous work. However, the important point is that the increase was similar in the two kinds of beef.

There was little difference, between the two kinds of beef, in total reducing substances and only a small increase as a result of a longer ripening period. Sulfydryl values increased between the 15.7- and 50-day intervals somewhat more rapidly in the beef from grain-fattened cattle, with the result that there was less difference between the two kinds of beef after 50 days than after 15.7 days. During ripening, labile sulfur from the protein molecule splits off and is liberated as H_2S and other volatile compounds. The sulfydryl is probably derived from cystine, glutathione, and other sulfur-bearing amino acids that are liberated during autolysis.

With respect to the content of amino and nonprotein nitrogen (the indices of protein break-down) as well as total nitrogen, the differences between the two kinds of beef were found to have no statistical significance either for the 15.7- or the 50-day ripening period. Sulfydryl values, on the other hand, were significantly higher for the beef from grass-fattened cattle after 15.7 days of ripening, owing probably to the higher sulfydryl content of the grass ration. After 50 days, however, the difference was not significant. The latter

TABLE 1.—*Comparative chemical composition and percentage of expressible juice of beef from grain-fattened and grass-fattened steers after a short (an average of 15.7 days) and a long (50 days) period of ripening*

SHORT PERIOD OF RIPENING

Item		1937-38			1938-39			1939-40			3-year average		
		Grain	Grass	Difference	Grain	Grass	Difference	Grain	Grass	Difference	Grain	Grass	Difference
Animals	number	3	3	----	3	3	----	3	3	----	9	9	----
Ripening period	days	18	18	----	16	16	----	13	13	----	15.7	15.7	----
Moisture	percent	72.73	73.15	-0.42	75 62	73 39	+2 23	72 54	72.28	+0.28	73 63	72.93	+0.6967
Ash	do	1.087	1.110	+.023	1 067	1 050	+.017	1.200	1.190	+.010	1.1177	1.1167	+.0010
Protein	do	23 12	22 39	+.73	21 81	22 72	+.91	22 47	22.74	-.27	22 47	22.62	-.1500
Ether extract	do	4 62	4.67	+.05	2 50	3 05	-.55	3 26	3.97	-.71	3 46	3.90	-.4367
Total nitrogen	do	3.70	3.58	+.12	3 49	3 58	-.09	3.60	3.64	-.04	3 60	3 60	-.0033
Nonprotein nitrogen	do	.388	.401	-.013	.419	.418	+.001	.408	.421	-.013	.405	.413	-.0083
Nonprotein nitrogen as related to total nitrogen	percent	10 50	11 21	-.71	11 99	11 67	+.32	11 33	11.58	-.25	11 28	11.49	-.213.
Amino nitrogen	do	.0689	.0702	-.0013	.0601	.0596	+.0005	.0558	.0550	+.0008	.0616	.0616	0
Amino nitrogen as related to total nitrogen	percent	1 86	1.96	-.10	1.72	1 66	+.06	1 55	1.51	+.04	1.71	1.71	0
Total reducing substances	do	1.567	.1687	-.0120	.0597	.0587	+.0010	.1547	.1523	+.0024	.1237	.1266	-.0029
Sulfur[dry]	do	.0303	.0560	-.0257	.0300	.0433	-.0133	.0312	.0441	+.0129	.0305	.0478	-.0173
Protein, fat-free	do	23 85	23.16	+.69	22 14	23 38	-1 24	23 39	23.64	-.25	23.13	23 39	-.2667
Ratio of moisture to protein	do	3 15:1	3 27:1	-12:1	3 47:1	3 23:1	+.24:1	3 23:1	3 18:1	+.05:1	3 28:1	3 22:1	+.057:1
Expressible juice	do	34 70	35 96	-1 26	40.20	39 70	+.50	44 80	48.50	-3 70	39 90	41.39	-1 49

LONG PERIOD OF RIPENING

Item		1937-38			1938-39			1939-40			3-year average		
		Grain	Grass	Difference	Grain	Grass	Difference	Grain	Grass	Difference	Grain	Grass	Difference
Animals	number	3	3	----	3	3	----	3	3	----	9	9	----
Ripening period	days	50	50	----	50	50	----	50	50	----	50	50	----
Moisture	percent	72.01	72.55	-0.54	72.59	72 31	+0.28	72 74	72.09	+0 65	72 45	72.32	+0.1300
Ash	do	1.110	1.077	+.033	1 060	1 120	-.060	1.127	1 063	+.064	1 0989	1.0867	+.0122
Protein	do	23 43	22 52	+.91	23 63	24 20	-.57	22 98	22.81	+.17	23 35	23.18	+.1700
Ether extract	do	2.99	3 77	-.78	2 58	2 47	+.11	2 42	3 07	-.65	2 66	3 10	-.4400
Total nitrogen	do	3.75	3 60	+.15	3 78	3 87	-.09	3 68	3 65	+.03	3.74	3 71	+.0300
Nonprotein nitrogen	do	.525	.504	+.021	.505	.505	0	.533	.515	+.018	.521	.508	+.0130
Nonprotein nitrogen as related to total nitrogen	percent	14 00	13 98	+.02	13 34	13 02	+.32	14 51	14 11	+.40	13 95	13.70	+.2467
Amino nitrogen	do	.0366	.0981	-.0015	.1024	.0989	+.0035	.1019	.1029	-.0010	.1003	.1000	+.0003
Amino nitrogen as related to total nitrogen	percent	2 58	2.73	-.15	2 71	2 55	+.16	2 77	2 82	-.05	2 68	2.70	-.0200
Total reducing substances	do	.3640	.3690	-.0050	.6363	.6413	-.0050	.0790	.0697	+.0093	.1598	.1600	-.0002
Sulfur[dry]	do	.1050	.1087	-.003	.0664	.0728	-.0064	.0778	.0938	-.016	.0831	.0918	-.0087
Protein, fat-free	do	24 27	23 42	+.85	21 30	24 79	-.49	23 73	23 77	-.04	24 10	23 99	+.1067
Ratio of moisture to protein	do	3 07:1	3 22:1	-.15:1	3 07:1	2 99:1	+.08:1	3 17:1	3 16:1	+.01:1	3 10:1	3 12:1	-.02:1
Expressible juice	do	31.70	31 60	+.10	38 90	42 00	-3 10	47 00	46 60	+.40	39 20	40.06	-.86

finding indicates that ripening may produce volatile sulfydryl compounds and that some loss results. Except for sulfydryl, the small differences obtained in these constituents are believed to be within the range of experimental error.

After an average ripening period of 15.7 days, no difference was observed in the flavor and aroma of the cooked seventh-eighth-rib cuts from the two kinds of meat. After 50 days, the characteristic flavor and aroma of ripened beef were rather pronounced, but again no difference in flavor and aroma between the grain-fattened and grass-fattened beef was detected. The results after both short and long periods of ripening indicate that the expressible-juice content of the beef from grass-fattened cattle was not significantly different from that of the grain-fattened steers.

SUMMARY AND CONCLUSIONS

A 3-year study was carried on in 1937–40 at the United States Department of Agriculture, Beltsville Research Center, Beltsville, Md., to compare the ripening of meat from steers about 2½ years old fattened on bluegrass pasture with that from cattle fattened on corn, cottonseed meal, and hay when the two kinds of beef are of equal fatness. Rib cuts from 18 paired carcasses, representative of a total of 120 animals, were used during the 3 years. Pair mates of as nearly the same fat content of eye muscle as possible were selected. One sixth-seventh-eighth-rib cut from each carcass was ripened at 33°–36° F. for an average of 15.7 days; the other, for 50 days.

No significant difference was found in the rates of ripening of the beef from the two types of feeding. This result was shown chemically by data on basic composition and especially by determinations of amino and nonprotein nitrogen. The sulfydryl content of beef from grass-fattened cattle was the higher but had no bearing on the rate of ripening, inasmuch as the indexes of protein break-down were similar for beef from both types of feeding. The higher sulfydryl content of beef from grass-fattened cattle is probably due to the higher content of sulfydryl of the grass ration.

No difference was observed in the flavor and aroma or in the expressible-juice content of the two kinds of beef.

DESIGN OF EXPERIMENTAL COMPARISONS BETWEEN LINES OF BREEDING IN LIVESTOCK [1]

By R. E. Comstock, *research instructor in animal husbandry*, and L. M. Winters, *professor of animal husbandry, Division of Animal and Poultry Husbandry, Minnesota Agricultural Experiment Station*

INTRODUCTION

The comparison of animals representing different breeding is one of the most common forms of experimentation in the field of animal improvement. The characteristics for which they may be compared are many. Present emphasis on the development of selected inbred lines will result in a great increase in this type of experimentation if the possible uses of these new lines to the livestock industry are to be accurately evaluated. Large numbers of experimental comparisons between the various lines, the breeds in general, breed crosses, and crosses of lines both within and between breeds will be necessary if the most productive lines and crosses are to be identified. This type of work is and will remain subject to serious financial limitations because of the requirements for space, labor, and equipment. Therefore, it is highly important to make the most efficient possible use of experimental animals.

The purpose of this paper is to clarify the effects that varying numbers of males and females used to produce progenies will have on the efficiency with which experimental comparisons of such progenies can be made. A specific objective will be to answer questions of the following type: Will a comparison of two lines be made more efficiently by use of the offspring, in each line, of 2 sires bred to 10 females each or of 3 sires bred to 5 females each? The term "line" will be construed to include inbred lines, crosses between separate inbred lines or breeds, and the various breeds in general.

ELABORATION OF THE PROBLEM

The most efficient experiment for the comparison of two lines will be defined as the one that allows the smallest observed difference to be judged of sufficient size to indicate a real difference between the lines in question. The significance attached to any experimental difference is based on its size in relation to the random variation of differences of the same kind in repetitions of the experiment. Other things being equal, a difference between the means of 2 sets of 10 animals would always be considered more significant than a difference of the same size between the means of 2 sets of 5 animals because, being based on more animals, it would be less likely to have resulted from random variation.

Consider now the number of males and females to be used in producing samples of lines for comparison. It is clear that increasing the progeny of a single sire by breeding more females to him will increase

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the accuracy with which his breeding performance, when mated to females of a certain line, is measured. However, it is uncertain whether he is representative of his line and, therefore, whether his progeny adequately represents the line or cross being tested. Assurance that the sample is representative of the entire line will be attained only when the number of sires producing it is increased. Because the total number of experimental animals that can be raised is limited, it is important to know the balance between the number of progeny to be raised from each sire and the number of sires to use in each line which will give the most efficient experiment.

AN APPROXIMATE SOLUTION

DEFINITION OF DIFFERENCE NECESSARY FOR SIGNIFICANCE

A mathematical expression of efficiency (as herein defined) in terms of number of sires, dams, and progeny can be derived from the use of well-known statistical concepts.

The significance of a difference between the means of samples of animals from two different lines can be tested statistically by the use of "Student's" distribution, using the argument,

$$t = d/\sqrt{s_1^2 + s_2^2}, \tag{1}$$

wherein

$d =$ difference between the means of the two lines,

$s_1 =$ standard error of the mean of one line, and

$s_2 =$ standard error of the mean of the other line.

Then d, equal to $t\sqrt{s_1^2 + s_2^2}$, defines the minimum difference necessary for significance if the value of t is inserted that has the maximum probability of occurrence by chance to be accepted as indicating a significant difference.

The variance of the mean of k full sibs can be expressed as

$$\sigma_1^2 = K/k$$

where K is the variance of the full sibs. The variance of the mean of fk animals where k offspring are raised from each of f dams all bred to the same sire is

$$\sigma_2^2 = \frac{F + K/k}{f}$$

where F is the variance of the paternal half sibs resulting from differences in dams or differences in environment to which full sibs are not subjected. Analogically the variance of the mean of mfk animals where k offspring are raised from each of mf dams, f of which are bred to each of m sires, is

$$\sigma^2 = \frac{M + \dfrac{F + K/k}{f}}{m}$$

where M is the variance of the maternal half sibs resulting from sire differences.

Then

$$\sigma^2 = \frac{M}{m} + \frac{F}{mf} + \frac{K}{mfk}$$

$$= M\left(\frac{1}{m} + \frac{1}{mf} \cdot \frac{F}{M} + \frac{1}{mfk} \cdot \frac{K}{M}\right)$$

In the above the symbols, σ, M, F, and K indicate parametric values.

Let the experiment comparing two lines be symmetrical; i. e., the values of m, f, and k shall be the same for both lines. Assume that M has the same value in each of the lines being compared and that this is likewise true for F. This is justifiable only in the absence of definite knowledge to the contrary. One might presume that equality of either of these variances for two lines would only rarely occur, but lacking knowledge based on previous experience, one would not know which of two lines was the more variable. The assumption of equality would then be more logical than any other specific relationship. Under this condition K will also be essentially equal for the two lines. Then substituting in equation (1), remembering that on the average $s^2 = \sigma^2$,

$$d = t\sqrt{2M\left(\frac{1}{m} + \frac{1}{mf} \cdot \frac{F}{M} + \frac{1}{mfk} \cdot \frac{K}{M}\right)} \qquad (2)$$

$$= t\sqrt{2M\left[\frac{1}{m} + \frac{1}{mf}\left(\frac{F}{M} + \frac{K}{Mk}\right)\right]} \qquad (3)$$

The value, d, so defined, is the minimum difference that will be judged significant in the average case. Values of t for different probability levels are available in almost any text on statistical methods applicable to small samples. The ratios F/M and K/M can be estimated within certain limits. Equation (2) or (3) can, therefore, be used to approximate d for given values of m, f, and k. The fact that d will be given in terms of M will not impair its usefulness for comparative purposes. This is all that is necessary since the authors do not intend that the values of d determined in this way shall be used for any purpose other than that of comparing the efficiency of experimental designs varying in the values of m, f, and k.

APPROPRIATE VALUE OF t

Values of t which have a specified probability of occurrence by chance vary with the number of degrees of freedom. This raises the question of how to determine the correct value to insert in the equation when using it to determine relative efficiency of experiments of different forms. Fisher[2] states: "* * * the very same causes that produce our real error shall also contribute the materials for computing an estimate of it" and "* * * if causes of variation affect the real error in such a way as to make no contribution to our estimate, this estimate will be vitiated, and will be incapable of providing a correct statement as to the frequency with which our real error will exceed any assigned quantity * * *" It is obvious that apparent differences between lines will be affected by the accuracy with which the sires used represent the average of their lines. Thus variation between sires affects our real error and must, therefore, contribute to any valid estimate of it. It follows that a valid estimate

[2] FISHER, R. A. THE DESIGN OF EXPERIMENTS. 252 pp., illus. Edinburgh and London. 1935.

of the error of the difference must be based on the variation between the means of the progenies of different sires within each line. Hence, the associated degrees of freedom in a comparison of two lines will be two less than the sum of the number of sires used in the two lines. This is the basis on which the authors have proceeded in the calculation of minimum differences. Thus, for an experiment in which four sires are to be used in each of two lines to be compared, the degrees of freedom are six.

ESTIMATION OF VARIANCE RATIOS

Characteristics which might be considered in the type of experiment under discussion can for our purposes be conveniently grouped in three classes depending upon the source of variance among paternal half sibs. Both variance which is genetic in origin and variance arising from the same environmental sources that cause variance among full sibs will, in each of the three classes, contribute to the variance among paternal half sibs. These will be the sole sources of variance among paternal half sibs in the case of the first group of characters. This means that there is no maternal influence and that no differences between individuals result in any way from the fact that they are out of different females except as their genotypes differ because of genetic diversity of their dams. For characters of this type, which should include milk production, fertility, egg weight, wool production, growth rate or mature weight of poultry, etc., F equals M and F/M is 1 (characters such as broodiness in chickens known to be sex-linked must be excepted). Two methods are then available for estimating K/M. Data on the offspring of different females but the same male can be used to estimate K/F if there is more than one offspring from each female. Since F and M are equal, this will also be an estimate of K/M. For example, the first value in table 1 was calculated from an analysis of variance, presented in table 2, of data on the average egg weight of four pullets from each of seven hens all bred to the same cock. As the number of hens approaches infinity the mean square for hens approaches $kF+K$. The value, 1.1879 from table 2, is therefore an estimate of $kF+K$. The value, 0.6841, estimates K. Substituting,

$$1.1879 = 4F + 0.6841,$$
$$F = 0.1260, \text{ and}$$
$$\frac{0.6841}{0.1260} = 5.43, \text{ an estimate of } K/F.$$

The other method of estimating K/M is based on knowledge of the sources of variance, K and M. If G is the additively genetic variance (defined to embrace four times the fraction of the variance arising from gene interactions [3] which contributes to the correlation between half sibs) in a line and E is the variance among full sibs not additively genetic, it can be shown [3] that

$$M = \tfrac{1}{4} G,$$
$$K = \tfrac{1}{2} G + E, \text{ and}$$
$$\frac{K}{M} = \frac{\tfrac{1}{2} G + E}{\tfrac{1}{4} G} = 2 + 4E/G.$$

If, for example, G amounted to one-half of the total variance, it would be equal to E and K/M would be 6 for the character in question.

[3] WRIGHT, SEWALL. THE ANALYSIS OF VARIANCE AND THE CORRELATIONS BETWEEN RELATIVES WITH RESPECT TO DEVIATIONS FROM AN OPTIMUM. Jour. Genet. 30: 243–256. 1935.

TABLE 1.—*Values of K/F estimated from data on poultry, swine, and sheep*

[Unpublished data from the Minnesota Agricultural Experiment Station]

Class of livestock	Characteristic	Number of animals	Number of dams	K/F
Poultry	Egg weight	28 30 15 32 32 32	7 6 6 8 8 4	5. 43 1. 98 5 14 ∞ 22 48 ∞
Total or weighted average		169	38	[1] 13 97
Swine	Daily rate of gain from 50 to 200 pounds.	1, 257	226	[2] 5. 17
Sheep	Post-weaning growth (weight increase from 105 to 165 days of age).	178	89	10. 89

[1] Weighted average of K divided by weighted average of F. ∞ arises as an estimate of K/F when the estimate of $kF+K$ is less than that for K. Since F cannot actually be negative it is apparent that this circumstance has resulted from random variation. This is to be expected occasionally when small groups of data are being used. By grouping data which gave such results with other groups for which the number of offspring per dam was the same it was possible to make use of all the data and still get positive estimates of F in all cases.

[2] Calculated from intrafarrow, intrastation variance.

TABLE 2.—*Analysis of variance of the data used in obtaining the estimate of K/F in table 1*

Variance due to—	Degrees of freedom	Sum of squares	Mean square
Hens	6	7 1277	1 1879
Within hens	21	14 3660	6841
Total	27	21. 4937	

Characters of the second class, while subject to little or no direct maternal influence, are distinguished from those of the first class by the fact that there is more nongenetic variance among paternal half sibs than among full sibs. This may be partly due to residual maternal influence but is largely due to full sibs being exposed to a more uniform environment. For example, the pigs which furnished the data for the estimation of K/F presented in table 1 were farrowed over a period of 2 months in each of the different years. Climatic conditions would therefore be more uniform for pigs within any one litter than for pigs in different litters. Further, each litter was raised in a separate lot, with the result that pasture available would vary less within than between litters. This would be true as well for exposure to parasites or infections. Thus, it was observed that on a few occasions when mange appeared, it was distributed more uniformly within than between litters. For characters of this type F exceeds M. K/F can be estimated as before. K/M and F/M can be estimated from this estimate of K/F and a knowledge of the amount of genetic variance. Post-weaning growth rate of swine will be used as an example. If it is assumed that G is 32 percent of the total variance (Whatley [4] estimates between 30 and 40 percent for 180-day weight

[4] Unpublished data.

of swine, and unpublished data from this station yield an estimate of 30 percent for post-weaning growth rate)

$$M = 8 \text{ percent of total variance and}$$

$$F + K = 92 \text{ percent of total variance.}$$

Since

$$K/F = 5 \text{ (from table 1),}$$

$$K = \tfrac{5}{6} \times 92 \text{ percent} = 76.67 \text{ percent and}$$

$$F = \tfrac{1}{6} \times 92 \text{ percent} = 15.33 \text{ percent.}$$

$$K/M = \frac{76.67}{8} = 9.58$$

$$F/M = \frac{15.33}{8} = 1.92.$$

In cases where only one offspring is raised per female as in cattle, K/F cannot be estimated as described. However, it will be noted

that where k is 1, the term $\left(\dfrac{F}{M} + \dfrac{K}{Mk}\right)$ in (3) becomes $\left(\dfrac{F+K}{M}\right)$, which

is easily estimated from knowledge of G. Using the above figures,

$$\frac{F+K}{M} = \frac{92}{8} = 11.5.$$

The third class of characters, of which birth and weaning weights are typical, are those for which maternal influences cause a large amount of variance among paternal half sibs. In reality they measure characteristics of the female more than of the offspring. Thus weaning weight of pigs is more a measure of milk secretion of the sow than of inherent capacity for growth of the pigs. If measures of this kind are thought of as describing maternal characters they can be put in the first group and treated accordingly.

Table 1 contains estimates of K/F for three different characteristics in three classes of livestock. The estimate for growth rate of swine is based on enough animals to make it fairly reliable for the conditions under which the swine were raised. The separate estimates for small groups of data are presented in addition to the value for all data considered together in the case of egg weight to demonstrate the variation to which this ratio is subject when it is based on data from small numbers of animals.

CALCULATED VALUES OF D

Table 3 is presented to show how d varies with the number of males and females used in two specified situations. The value of t was used which had, for the appropriate number of degrees of freedom, a probability of 0.05 of occurring by chance.

TABLE 3.—*Differences (relative [1]) necessary for significance when comparing means of two lines for various values of m and f*

WHEN $F/M=2$, $K/M=10$, $k=5$

f	*m*						
	2	3	4	5	6	8	10
1	9.62	5.07	3.87	3.26	2.88	2.40	2.10
2	7.45	3.93	3.00	2.53	2.23	1.86	1.63
3	6.57	3.46	2.64	2.23	1 96	1.64	1.44
4	6.09	3.21	2.45	2.06	1.82	1.52	1.33
5	5.77	3.04	2.32	1.96	1.73	1.44	1.26
6	5 56	2.93	2.23	1.88	1.66	1.38	1.21
8	5.27	2.78	2.12	1.79	1.58	1.31	1 15
10	5.09	2.68	2.05	1.73	1.52	1.27	1.11
∞	4.30	2.27	1.73	1.46	1.29	1.07	.94

WHEN $\dfrac{F+K}{M}=19$ AND $k=1$

f	*m*						
	2	3	4	5	6	8	10
1	19.24	10.14	7.74	6.52	5.75	4 80	4.20
2	13.94	7.35	5 61	4.72	4.17	3.47	3.04
3	11 65	6.14	4 69	3.95	3.48	2.90	2.54
4	10.32	5.44	4.15	3 50	3.08	2.57	2.25
5	9.43	4.97	3.79	3 20	2.82	2 35	2.06
6	8.78	4.63	3 53	2 98	2.63	2.19	1.92
8	7.90	4.16	3.18	2.68	2.36	1 97	1.73
10	7.33	3.86	2.74	2.48	2.19	1.83	1.60
∞	4.30	2 27	1 73	1.46	1.29	1.07	.94

[1] $\sqrt{M}$ has been dropped from each of the tabled values.

The first value in table 3 was obtained as follows: $f=1$, $m=2$, $k=5$, $F/M=2$, $K/M=10$, and $t=4.303$ for two degrees of freedom (see (*b*) above) and $P=0.05$. Substituting in equation (3),

$$d=4.303\sqrt{2\left[\frac{1}{2}+\frac{1}{2}\left(2+\frac{10}{5}\right)\right]}\cdot\sqrt{M}=9.62\sqrt{M}.$$

The rest of the values in the first part of table 3 were obtained in the same way by substitution of the correct values of f and m. The same procedure was followed for the second part of table 3 except that k was set at 1 and $\left(\dfrac{F}{M}+\dfrac{K}{Mk}\right)$, which equals $\left(\dfrac{F+K}{M}\right)$ when k is 1, at 19.

Equation (3) makes it clear that whenever the term $\left(\dfrac{F}{M}+\dfrac{K}{Mk}\right)$ is constant the value of d for any specified values of m and f will be constant as well. For example, the values of d in table 3 were calculated to apply for post-weaning growth rate in swine, setting F/M at 2, K/M at 10, and k at 5. The term $\left(\dfrac{F}{M}+\dfrac{K}{Mk}\right)$ was then equal to 4. The tabled values would have been identical for a character for which F/M was 1, K/M 6, and k 2. The values in the second part of table 3 were calculated to apply to any case where the additively genetic portion of the variance is 20 percent and k is 1. Thus $\dfrac{F+K}{M}$ was set at 19. This table would have been the same for a situation where F/M was 1, K/M 36 (i. e., G equal to 10.5 percent of the total variance), and k 2.

Table 3 does not include cases for which but one sire is used in each line. The reason is that in such cases there are no differences between means of progenies of sires from which to estimate experimental error and, therefore, no precise method by which to determine whether an observed difference indicates a true dissimilarity between the lines or is no larger than might occur between the progenies of two sires from the same line.

Let it be assumed that pigs from 12 litters per line are to be raised for a comparison of two lines for post-weaning growth. From table 3 the differences necessary for significance if six females are bred to each of two males in each line, if four are bred to each of three males, if three are bred to each of four males, or if two are bred to each of six males are $5.56\sqrt{M}$, $3.21\sqrt{M}$, $2.64\sqrt{M}$, or $2.23\sqrt{M}$, respectively. As another example, assume that two lines of cattle fed individually are to be compared. Because of the work and equipment required only eight animals are to be fed from each line. If we assume the variance of efficiency in the feed lot is about 20 percent additively genetic, the values in the second part of table 3 can be used. Then if the eight animals are by two males, four males, or eight males, the difference necessary for significance will be $10.32\sqrt{M}$, $5.61\sqrt{M}$, or $4.80\sqrt{M}$, respectively.

DISCUSSION

Several general conclusions can be drawn from consideration of equation (2). First, whenever mf, total females used in each line, and mfk, total animals raised in each line, are constant an increase in m always decreases d. Further, when mfk is constant any increase in m or f compensated by decrease in k will decrease d, though increasing m will decrease d more than increasing f. These facts become more important with every limitation on the number of experimental animals. The requirement for a large amount of labor on each animal as in individual feeding or determination of carcass cutout value is a typical example. In such cases anything in the design of the experiment which will make the labor expended more fruitful is especially worth while.

When K/Mk is large either because of a smaller proportion of variance being additively genetic or because of a smaller value of k, the increase in efficiency resulting from the use of more sires is relatively less. The proportion of the variance of a specific character which is additively genetic may decrease as a result of either increased inbreeding of the line (or, in the case of crosses between lines, of the lines from which the parent stock is taken) or increased environmental variation. This emphasizes the importance of standardizing environment and making measurements as accurately as possible in all experiments of this type so as to minimize nongenetic variation. It is likely that in a well-conducted experiment the nongenetic portion of variance can be reduced to some extent from what it is in the average breeding herd. For example, the ratio K/F for growth in lambs (table 1) would probably have been smaller if the weight increase had been based on the average of two or three weights at the beginning and end of the 60-day period instead of on one weight at each time. However, while K/M will vary considerably depending on the characteristic in question, standardization of environment,

the inbreeding of the line or the inbreeding of lines used in producing crosses between lines, it should be remembered that the size of K/Mk affects only the degree to which d changes with f or m, not the direction in which it changes.

Table 4 has been presented for reference purposes. It contains some of the estimates of additively genetic variance that have been made up to the present time.

TABLE 4.— *Estimates of the additively genetic portion of variance*[1] *for various characteristics of dairy cattle and swine*

Class of livestock	Characteristic	Additively genetic variance
Dairy cattle	Butterfat production	[2] 0. 20-0. 25
	Post-weaning rate of gain	[3]. 24
	____do____	[4]. 30
Swine	180-day weight	[5] 30- .40
	Thickness of back-fat	[3] 47
	Thickness of belly	[5] 46
	Body length	[3]. 54

[1] Additively genetic variance estimated as 4 times the correlation between half sibs is equal to G as herein defined if environmental factors have not affected the correlation. Estimates made by doubling parent-offspring correlations or regressions of offspring on parent, subject to the same restriction regarding environmental factors, tend to overestimate G. There is, however, reason to believe that the amount of overestimation is usually slight.

[2] LUSH, JAY L. INTRA-SIRE CORRELATIONS OR REGRESSIONS OF OFFSPRING ON DAM AS A METHOD OF ESTIMATING HERITABILITY OF CHARACTERISTICS. Amer. Soc. Anim. Prod. Proc 33 293-301. 1940.

[3] LUSH, JAY L. GENETIC ASPECTS OF THE DANISH SYSTEM OF PROGENY-TESTING SWINE. Iowa Agr. Expt. Sta. Res. Bul. 204, pp. [105]-196, illus. 1939.

[4] Unpublished data from this station.

[5] Information to the authors from Dr. J. A. Whatley, Jr., of the Oklahoma Agricultural Experiment Station.

The question naturally arises whether variation among the progeny of different females could not be used in the estimation of experimental error in order to increase degrees of freedom and reduce the value of t required. To do this would imply that one was accepting the progeny of the sires actually used as being representative of the progeny of all possible sires in the particular lines compared. This would be correct only when genetic variance in a line was zero, in which case the progeny of different sires would all be alike genotypically. How much genetic variance could exist before a significant loss of precision would result from this procedure would be difficult to define. It would depend in part on the number of females used and the total number of animals raised as well as on the number of sires used. Further, one would not often have accurate knowledge of the magnitude of genetic variance. It appears that the most dependable conclusions will be reached if one takes full account of the possibility of differences between the progenies of different sires.

Experimental efficiency has been defined on the basis of a comparison of two lines. This is appropriate because many experiments will compare only two lines; and, even though several lines are being compared at one time, in the final analysis, one is interested in the difference between each line and each of the others.

SUMMARY

A formula for the estimation of the difference necessary for significance (and, therefore, experimental effiiency) in comparisons of lines of breeding has been presented. The smaller the difference necessary

for significance the more reliable is the observed difference as a criterion of the true difference between the lines. Therefore the calculated values of d represent experimental efficiency.

Tables have been compiled which demonstrate for specified conditions the effect on efficiency of varying numbers of males and females used to produce experimental animals.

Various relationships between numbers of breeding animals and efficiency have been pointed out and discussed. Because these relationships operate in all situations, though in varying degrees, their application is not limited to instances in which data for the estimation of the variance ratios in the formula are available.

In the design of any experimental comparison of lines of breeding, the cost of using extra sires must be balanced against the increased efficiency to be gained from their use. It appears certain that the use of at least three or four sires in each line will always be profitable in terms of information that can be obtained from the experiment. On the other hand, increases in the number of females used will be more effective if the number of sires is also increased so that the number of females bred to each male remains small.

COMPOSITION OF THE TOPS AND ROOTS OF THE TIMO-THY PLANT AT SUCCESSIVE STAGES OF GROWTH [1]

By MAX PHILLIPS, *senior chemist*, B. L. DAVIS, *junior chemist*, and H. D. WEIHE, *assistant chemist, Agricultural Chemical Research Division, Bureau of Agricultural Chemistry and Engineering, United States Department of Agriculture*

INTRODUCTION

The chemical composition of certain crop plants at different stages of growth and development has been the subject of several contributions from this laboratory (*5, 18, 19, 20*).[2]   Those studies were undertaken for the purpose of determining the relative and absolute quantities of the structural and nonstructural components of the plant and to ascertain their various interrelationships.

The study reported here was undertaken to determine the chemical composition of the tops and roots [3] of the timothy plant at successive stages of its development.   The results are calculated both on a percentage and an absolute basis.   It is believed that in this way a better understanding may be obtained of the possible precursors and interrelationships of the several components of the plant.

REVIEW OF LITERATURE

There is, at present, an extensive literature on the chemical composition of grasses in general and of timothy in particular.   For the most part the analytical results have been obtained by conventional methods and are reported in the usual terms, such as crude protein, crude fiber, and nitrogen-free extract.   The review presented here is limited to publications of particular interest to the present study.

Sixty-three years ago (1878) Vasey and Collier (*22*) of the United States Department of Agriculture made an extensive study of the chemical composition of many grasses and forage plants.   They analyzed the ash of these plants and also determined the more important organic constituents.   Among the latter, they reported the percentages of oil, wax, sugars, gum and dextrin, cellulose, "amylaceous cellulose," alkali extract, and albuminoids.   Collier (*3*), in his annual report to the United States Commissioner of Agriculture for the year 1879, gives additional data on the composition of American grasses.

Vasey and Richardson (*23*) in 1884 published the results of an extensive study of the chemical composition of American grasses. Their results on the percentages of the organic components of the various grasses were reported in conventional terms, such as fat, nitrogen-free extract, crude fiber, albuminoid, nonalbuminoid, and total nitrogen.   They also reported on the chemical composition of 15 grasses at different stages of maturity.   In analyzing timothy, they took samples at five different periods of development.   They found that the percentage of ash, albuminoid, nonalbuminoid, and

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[1] Received for publication October 10, 1941.
[2] Italic numbers in parentheses refer to Literature Cited, p. 544.
[3] The term "roots" as used in this paper includes the entire subterranean part of the plant, including the bulbs.

total nitrogen decreased, whereas the fat, nitrogen-free extract, and crude fiber increased, as the plant developed and matured.

Frear, Carter, Browne, Brooks, and Norris (9) in 1904 reported the results of a systematic analysis of timothy hay that had been cut when well past bloom. The scheme of analysis used was a modification of that proposed by Dragendorff (6) and by Parsons (16). The several fractions obtained were subjected to a systematic examination, and the residual plant material containing the hemicelluloses was hydrolyzed with 6-percent sulfuric acid. Both xylose and arabinose were identified in the hydrolysate. The lignin (lignic acid) content of the hay was found to be 9.6 percent.

Haigh (11) collected timothy plants at different stages of growth. The plants were separated into four parts, namely, heads, stalks with leaves, stubble, and bulbs. The results indicated that the heads increased in dry matter throughout the growth and ripening period. The nitrogen-free extract of the heads increased at a greater rate than all the other constituents. The nitrogen-free extract and crude fiber of the stalks and leaves increased during growth and ripening. The nitrogen and ether-soluble matter of the stalks and leaves increased during growth but decreased to some extent during ripening.

Hunt, Record, and Bethke (13) found that the protein of timothy decreased and the crude fiber increased as the plant matured. Similar results were reported by Evans and Thatcher (8).

Hosterman and Hall (12) harvested timothy at five different stages of development, beginning when the plants were nearly fully headed and ending when they were fully mature. The results for the whole hay indicated that the percentage of crude protein decreased, whereas the percentage of crude fibre increased, as the plant developed and matured.

MATERIAL AND METHODS OF ANALYSIS

The timothy plants (*Phleum pratense L.*) used in this investigation were collected from a large field at the United States Department of Agriculture Research Center at Beltsville, Md.[4] The first harvest took place in the morning of May 8. Subsequent harvests, with the exception of the last one, which occurred 4 days after the previous one, were made at weekly intervals. The plants were taken up by the roots, freed of as much soil as possible, and quickly transported to the laboratory and counted. The roots were then cut off and washed repeatedly in running water to remove any adhering soil. The tops and roots were dried at 60° C. in a large steam drier, then spread out in the laboratory, air-dried, and weighed. The air-dried tops and roots from each harvest were ground in a Wiley mill fine enough to pass through a 60-mesh sieve. Separate samples were taken for moisture and ash determinations, and the analytical data were calculated on the moisture-free and ash-free basis.

The various determinations, except those indicated below, were made as described in a previous publication (20).

Hot water plus 1-percent hydrochloric acid extractives.—The determination with hot water and 1-percent hydrochloric acid was carried

out as described in a previous publication (*20*) except that no attempt was made to determine separately the hot water and the 1-percent hydrochloric acid extractives.

Crude cellulose.—Crude cellulose was determined by the method of Kürschner and Hanak (*14*).

Lignin.—Lignin was determined by the method of Goss and Phillips (*10*) except that no correction for nitrogen was made. The results are reported as percentage of ash-free crude lignin.[5]

Pectic substances.—The plant material was extracted with a hot 0.5-percent ammonium oxalate solution. The pectic substances in the ammonium oxalate extract were precipitated with acidified ethanol and determined as calcium pectate by the method of Carré and Haynes (*2*). The following is a detailed description of the method used:

The weighed sample (10 or 15 gm.) was placed in a 250-cc. centrifuge bottle containing 150 cc. of aqueous 0.5-percent ammonium oxalate solution (when a 15-gm. sample was taken, 200 cc. of the ammonium oxalate solution was used). The mixture was heated to 85° C. in a water bath, the bottle was stoppered tightly with a rubber stopper, which was securely fastened with a brass holder attached to the neck of the bottle. The bottle was placed in a large water bath provided with an electric motor, immersion heaters, constant temperature control, and a device for holding and rotating endwise 10 bottles at one time, and was heated at 85° for 1.5 hours. The bottle and contents were centrifuged, the clear supernatant solution was decanted, and a few cubic centimeters of toluene was added to the extract to check any microbial decomposition. To the residue in the centrifuge bottle 100 cc. (150 cc. with a 15-gm. sample) of fresh 0.5-percent aqueous ammonium oxalate solution was added, the mixture was well shaken and centrifuged, and the clear supernatant solution was added to the first ammonium oxalate extract. The residual material was again digested for 1.5 hours at 85° with 100 cc. (150 cc. with a 15-gm. sample) of 0.5-percent aqueous ammonium oxalate solution and again washed with the ammonium oxalate solution as already described. This operation was repeated three times more, making a total of five successive digestions at 85° and five washings. The extracts and washings were combined, filtered through a Gooch crucible, and concentrated on the steam bath to a volume of 80 cc. (120 cc. with a 15-gm. sample). To the concentrated solution 3.5 volumes of 95-percent ethanol containing 5 cc. of concentrated hydrochloric acid per liter was added, and after it had stood overnight, the precipitate of crude pectin was separated with the aid of the centrifuge. The crude pectin was shaken with 80-percent ethanol containing 5 cc. of concentrated hydrochloric acid per liter [6] and centrifuged. The supernatant liquid was discarded. This operation was repeated until the alcoholic washings no longer gave a test for oxalic acid or oxalate. The pre-

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[5] In determining the percentage of lignin in the roots the following modification was introduced: A 2-gm. sample was weighed out, and this was successively extracted with a 1·2 alcohol-benzene solution and boiling 1-percent hydrochloric acid solution, the procedure outlined in the paper (*10*) already referred to being followed. The extracted sample was collected on fine quantitative filter paper and after a small hole had been punctured in the paper, the plant material was washed quantitatively with water into the large test tube used for the hydrolysis with the fuming hydrochloric acid. The tube was placed on the steam bath, and the mixture was evaporated to dryness. Fuming hydrochloric acid was added to the dry residue, and the determination was completed as prescribed by Goss and Phillips (*10*).

[6] Subsequently it was found that washing the crude pectin precipitate with 80-percent ethanol containing 10 cc. of concentrated hydrochloric acid per liter resulted in a quicker removal of the oxalic acid and oxalate.

cipitate was dissolved in 50 cc. of hot water containing 2 cc. of concentrated ammonium hydroxide solution, and the solution was filtered through a Hirsch type sintered glass funnel of fine porosity. The centrifuge bottle was washed with two 50-cc. portions of hot water, the washings were poured through the Hirsch funnel previously used, and the filtrate was collected in the same beaker containing the solution of the pectic substances. The combined filtrate and washings, after cooling to room temperature, were treated with 100 cc. of $\frac{N}{10}$ (approximately) sodium hydroxide solution and allowed to stand overnight. To this alkaline solution, 50 cc. of $\frac{N}{1}$ (approximately) acetic acid and an equal volume of $\frac{M}{1}$ (approximately) calcium chloride solution were added, and the whole was boiled for 10 minutes. The gelatinous calcium pectate precipitate was collected on a weighed filter paper, washed with hot water until free of chlorides, dried at 105° and weighed. The result was calculated as percentage of calcium pectate.

RESULTS

The results obtained are recorded in tables, 1, 2, and 3. Table 1 shows the percentage composition of the timothy plant at successive stages of growth; table 2 shows the percentages of furfural yielded by several components of the plant; and table 3 gives the weights of the various constituents.

ASH

The percentage of ash in the tops increased from 8.88 in the first harvest period to 9.69 in the second. Subsequently, there was a general, although somewhat irregular, decrease as the plants grew older. The percentage of ash in the roots varied in a rather irregular manner. This was undoubtedly due to the fact that in spite of repeated washings, it was very difficult to free the roots entirely of sand and other inorganic matter.

NITROGEN AND CRUDE PROTEIN

With the exception of the plants from the last harvest period, the percentage of nitrogen and crude protein in the tops decreased generally as the plants grew older. This is in agreement with the results previously obtained by Phillips and coworkers (*18, 19, 20*) in their studies of wheat, barley, and oat plants at successive stages of growth. The percentage of nitrogen and crude protein in the roots decreased in the early stages of growth of the plants, reaching a minimum in the fifth harvest period, after which there was no significant change in percentage.

METHOXYL

The percentage of methoxyl in the tops increased regularly as the plants grew older. This regular increase is explained, in part at least, by the fact that in general the lignin content increases with the increase in the age of the plant. The methoxyl group is found in many substances of vegetable origin and forms an integral part of the lignin complex. There was no appreciable variation in the percentage of methoxyl in the roots of the timothy plant at successive stages of its development.

TABLE 1.—Composition[1] of timothy plant at successive stages of growth

Harvest No.	Ash	Nitrogen	Crude protein (N×6.25)	Meth-oxyl	Alcohol-benzene extractives	Hot water plus 1-percent hydrochloric acid extractives	Uronic acid (as anhydride)	Total furfural	Pento-sans	Crude cellulose	Furfural in crude[2] cellulose	Lignin[3]	Nitrogen in ash-free crude[4] lignin	Meth-oxyl in ash-free crude lignin	Pectic substances (as calcium pectate)
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
TOPS															
1	8.88	1.71	10.68	1.22	21.53	46.10	7.02	10.75	15.76	24.9	6.13	5.98	3.78	5.33	1.57
2	9.69	1.52	9.50	1.23	20.46	48.05	6.28	10.75	16.04	23.9	5.78	5.81	3.30	5.92	1.51
3	9.94	1.18	7.37	1.34	18.20	46.98	6.14	11.62	17.60	26.6	5.63	6.20	3.25	6.82	1.48
4	6.88	1.22	7.62	1.49	18.32	45.20	5.78	13.00	20.07	28.5	6.51	6.66	3.12	7.60	1.32
5	6.14	1.16	7.25	1.62	15.97	46.66	5.90	12.82	19.73	29.2	6.54	7.35	3.02	8.48	1.38
6	5.09	.89	5.56	2.11	14.06	45.22	4.88	14.37	22.76	31.4	5.97	8.52	1.86	12.24	.86
7	5.62	.86	5.37	2.22	10.69	49.84	5.24	13.80	21.65	30.4	6.69	8.47	2.20	11.98	.88
8	5.53	1.07	6.69	2.41	10.28	47.46	4.78	14.44	22.91	32.9	6.28	8.05	2.18	14.16	1.00
ROOTS															
1	12.04	0.90	5.62	1.59	14.67	53.99	7.60	16.90	25.90	31.7	4.84	8.96	2.03	7.00	1.95
2	22.22	.70	4.37	1.63	16.00	54.81	7.05	19.34	30.45	28.4	4.30	8.33	1.21		1.62
3	12.24	.59	3.69	1.60	16.55	54.13	7.11	16.86	26.18	28.8	4.86	7.98	1.42	8.03	1.39
4	12.92	.49	3.06	1.56	14.88	58.55	5.89	15.03	23.51	26.7	4.95	6.88	1.29	9.52	1.18
5	12.27	.45	2.81	1.53	15.49	61.16	5.76	13.83	21.51	24.2	5.03	6.97	1.33	8.81	1.10
6	10.51	.45	2.81	1.48	12.08	62.71	5.62	12.64	19.53	23.7	4.87	6.99	.87	9.36	1.03
7	10.08	.50	3.12	1.42	9.88	68.87	5.18	11.90	18.42	21.9	4.71	6.34	1.35	10.06	.79
8	9.88	.57	3.56	1.68	11.26	64.20	5.45	13.83	21.63	25.1	5.22	7.98	1.48	9.75	1.17

[1] All results, except the percentage of ash, were calculated on the moisture-free and ash-free basis. The percentage of ash was calculated on the moisture-free basis.
[2] Calculated as percentage of ash-free crude cellulose.
[3] Not corrected for nitrogen.
[4] Calculated as percentage of crude lignin.

ALCOHOL-BENZENE EXTRACTIVES

The percentage of alcohol-benzene extractives in the tops generally decreased as the plants grew older. In the roots, however, the results were rather irregular.

HOT-WATER AND 1-PERCENT HYDROCHLORIC ACID EXTRACTIVES

The fraction removed from the plant by successive extraction with hot water and with hot 1-percent hydrochloric acid consisted of a heterogeneous class of compounds, such as sugars, starch, hemicelluloses, and proteins. This explains, at least in part, the irregular results obtained in the analysis of the tops and roots of the plants.

URONIC ACIDS

In general the percentages of uronic acids in the tops and roots decreased, although not always regularly, as the plants grew older. These sugar acids are components of two important plant constituents, namely, the pectic substances and the hemicelluloses. As the plants grow older, the percentage of pectic substances, particularly in lignified tissues, decreases, while the percentage of hemicelluloses generally increases. However, as the pectic substances contain a much greater percentage of these acids than the hemicelluloses, a decrease in the percentage of pectic substances, unless accompanied by a much greater increase in the percentage of hemicelluloses, would lower the percentage of uronic acids in the plant material. This explains the general trend of the percentages of uronic acids in the tops and roots of the plants.

TOTAL FURFURAL

The furfural obtained when a plant material is distilled with 12-percent hydrochloric acid is derived from the uronic acids and from the pentoses or pentosans. These two groups of subtances are found in the gums, pectins, and hemicelluloses. A pentosan fraction also occurs in close association with the cellulose. Inasmuch as these components do not yield the same percentages of furfural when distilled with 12-percent hydrochloric acid and since some of them increase while others decrease as the plant develops and grows to maturity, the percentages of total furfural in the table represent an additive effect of several factors of unequal magnitude.

The percentage of total furfural yielded by the tops generally showed an upward trend as the plant grew and developed. This increase was not consistent, however, as is evident from the results obtained from the samples of the fifth and seventh harvests. The percentage of total furfural in the roots increased at first, reaching a maximum in the sample from the second harvest period, after which, with the exception of the roots from the last harvest period, there was a gradual decrease.

PENTOSANS

The term "pentosans" in this paper does not denote, as it usually does, the percentage of total furfural calculated as pentosans. Here it represents the difference between the total furfural and furfural derived from the uronic acids, calculated as percentage of pentosans. A description of the method used for obtaining the percentage of pentosans and a full discussion of the significance of this determination are given in a previous paper (*20*).

The percentage of pentosans in the tops increased steadily until the fifth harvest period, when there was a decrease. Subsequently it increased again, although not regularly, as the plants grew older. The percentage of pentosans in the roots was rather irregular, although the general trend was downward.

CRUDE CELLULOSE AND FURFURAL FROM CRUDE CELLULOSE

The percentage of crude cellulose in the tops generally increased, although not regularly, as the plants grew older. Thus in the plants from the first harvest period the percentage of crude cellulose was 24.9, whereas in those from the last harvest period it was 32.9. The percentage of crude cellulose in the roots was irregular. From an initial percentage of 31.7 it decreased to 21.9 in the seventh harvest period, and then it increased to 25.1 in the eighth.

The percentage of furfural yielded by the crude cellulose of both tops and roots showed no significant change during the development of the plants.

CRUDE LIGNIN, NITROGEN, AND METHOXYL IN CRUDE LIGNIN

In the values for lignin recorded in table 1, no correction was made for the nitrogen in the lignin. As was pointed out by Phillips (17) in the determination of the lignin content of materials rich in protein, it is not possible to apply a suitable correction for the nitrogenous complexes in the lignin because of the variability in the ratio between the increase of crude lignin and the increment of nitrogen in this material.

The percentages of crude lignin, nitrogen, and methoxyl in the crude lignin of the tops show the same general trend as those previously found in studies on wheat, barley, and oat plants (18, 19, 20), that is, the percentages of crude lignin and methoxyl in the lignin increased, in the main, while the percentage of nitrogen in the crude lignin decreased as the plants grew older. The percentages of crude lignin in the roots showed no significant variation, while the percentages of methoxyl in the crude lignin showed a slight trend upward as the plants grew older. The percentages of nitrogen in the crude lignin of the roots were rather irregular.

PECTIC SUBSTANCES

Three types of pectic substances are now recognized, namely, protopectin, pectin, and pectic acid. However, the results in the last column of table 1 represent only the total or sum of these pectic complexes in the tops and roots at different stages of growth of the plants.

The percentages of pectic substances (as calcium pectate) in both the tops and roots showed a general trend downward as the plants grew older, except for the last harvest. Bennett (1) in studying the composition of the tops of red clover (*Trifolium pratense*) noted a slight decrease in the percentage of pectic substances as the plants grew older. In Kentucky bluegrass (*Poa pratensis*), however, he observed no such relationship.

FURFURAL YIELDED BY SEVERAL COMPONENTS OF THE TIMOTHY PLANT

Table 2 shows the percentages of furfural yielded by several components of the tops and roots of the timothy plant at successive stages of growth. In this table an attempt was made to separate the percentages of furfural yielded by several components of the tops and

roots, thus affording a more accurate picture of the development of the furfural-yielding components. The figures in the second column are taken from table 1 and are inserted here for the sake of completeness and comparison. The figures in the third column were obtained by dividing the percentages of uronic acid anhydride recorded in table 1 by 4.60, the derivation of which is explained fully in a previous paper from this laboratory (*20*). The figures in the fifth column were obtained by multiplying the percentage of crude cellulose by the percentage of furfural in the crude cellulose (using the corresponding data in table 1) and dividing the result by 100. The figures in the seventh column were obtained by subtracting the sum of the percentages in the third and fith columns from the percentage of total furfural in the second column. The figures in the last column were obtained by multiplying the percentage of furfural from the pentoses of the polyuronides (seventh column) by 1.736. (The factor 1.736 was used because xylose when distilled with 12-percent hydrochloric acid, in accordance with the usual procedure, yields only 90 percent of the theoretical quantity of furfural).

TABLE 2.—*Furfural yielded by several components of the timothy plant*

[Calculated on basis of moisture-free and ash-free plant material]

TOPS

Harvest No.	Total furfural	Furfural from —						
		Uronic acids		Crude cellulose		Pentoses of polyuronides		Pentoses of polyuronides calculated as xylose
	Percent	*Percent*	*Percent of total*	*Percent*	*Percent of total*	*Percent*	*Percent of total*	*Percent*
1	10.75	1 53	14 23	1.53	14.23	7.69	71.54	13.35
2	10 75	1.37	12 74	1.38	12 84	8 00	74.42	13.89
3	11.62	1.33	11 45	1.50	12.91	8.79	75.64	15 26
4	13.00	1.26	9 69	1.86	14.31	9.88	76.00	17.15
5	12 82	1.28	9.98	1.91	14.90	9.63	75.12	16.72
6	14 37	1 06	7.38	1.87	13.01	11.44	79.61	19.86
7	13.80	1.14	8 26	2.03	14.71	10.63	77.03	18.45
8	14.44	1 04	7.20	2.07	14.33	11.33	78.47	19.67

ROOTS

1	16.80	1.65	9 82	1.53	9 11	13.62	81.07	23.64
2	19.34	1.53	7.91	1.22	6.31	16.59	85 78	28.80
3	16.86	1.55	9.19	1.40	8.30	13.91	82.51	24.15
4	15.03	1.28	8 52	1 32	8.78	12.43	82.70	21.58
5	13.83	1 25	9.04	1.22	8.82	11.36	82.14	19.72
6	12.64	1.22	9.65	1.15	9 10	10.27	81.25	17.83
7	11.90	1.13	9.50	1.03	8.65	9.74	81.85	16.91
8	13.83	1.18	8.53	1.31	9.47	11 34	82.00	19.69

Table 2 shows that the percentages of furfural yielded by the uronic acids of the tops decreased generally as the plants grew older. Thus, the uronic acids of the tops of the young plants furnished 14.23 percent of the total furfural, while those of the more mature plants furnished only 7.20 percent. Similar results were obtained in previous studies with the barley plant (*19*). The percentages of furfural yielded by the uronic acids of the roots, calculated as percentage of the plant material, showed a regular downward trend as the plants

grew older. When these were calculated as percentage of the total furfural, however, the decrease was not particularly striking. The percentages of furfural yielded by the crude cellulose of the tops varied only slightly as the plants grew older. The percentages of furfural yielded by the crude cellulose of the tops when calculated as percentage of the total furfural ranged from 12.84 to 14.90. The percentages of furfural from the crude cellulose of the roots were rather irregular. The percentages of furfural yielded by the pentoses of the polyuronides of the tops increased, though not regularly, as the plants grew older. In the roots, the percentages of furfural yielded by the pentoses of the polyuronides increased in the early stages of the development of the plant, then it decreased, and in the last harvest period it increased again. In all stages the greatest percentage of the total furfural was furnished by the pentoses of the polyuronides. The percentages ranged from 71.54 to 85.78. The figures in the last column, of course, show the same tendency as those in the seventh column.

WEIGHTS OF PLANTS AND WEIGHTS OF VARIOUS CONSTITUENTS AT DIFFERENT STAGES OF GROWTH

Table 3 shows the weights of the tops, of the roots, and of the more important constituents at different stages in the development of the timothy plant, all calculated on the basis of 100 plants.

The weights of the moisture-free matter in the tops and roots of the plants require no special comment. There was a regular and rapid increase in the weights of the tops as the plants grew and developed. The weights of the roots also increased regularly with the increase in the age of the plants, although, of course, not at the same rate or to the same extent as the tops.

The crude protein of the tops increased as the plants developed and approached maturity. The decrease from 1.39 gm. of crude protein in the first harvest period to 0.98 gm. in the third harvest period is undoubtedly due to some error in manipulation. The increase in crude protein in the tops was nearly 4.9 times as great in the last harvest period as in the first. The quantity of crude protein in the roots showed little variation in the early stages of the development of the plants, but in the later stages there was a pronounced increase.

The methoxyl in the tops and in the roots increased regularly as the plants grew and developed. Here, also, both the absolute and the relative increase was greater in the tops than in the roots.

The quantities of the various extractives (columns 8 and 9) in both the tops and roots increased steadily, although not uniformly, with the increase in the age of the plants.

The quantities of the uronic acids in the tops and in the roots increased steadily, with only two insignificant exceptions, as the plants developed and matured.

The pentosans, crude cellulose, lignin, and pectic substances may conveniently be considered together. In the tops all these four constituents increased steadily, with one slight exception, as the plants grew older. These results are of particular significance from the standpoint of the genetic relationship of these four plant constituents. Various theories as to the possible precursors of lignin have been proposed.

TABLE 3.—*Weight of 100 timothy plants and total weight of various constituents at successive stages of growth (1939)*

[Calculated on basis of moisture-free and ash-free plant material]

Harvest No.	Date harvested	Height of plants	Plants harvested	Weight of 100 plants (moisture-and ash-free basis)	Weight of various constituents in 100 timothy plants at successive stages of growth								
					Crude protein	Methoxyl	Alcohol-benzene extractives	Hot water plus 1-percent hydrochloric acid extractives	Uronic acids (as anhydrides)	Pentosans	Crude cellulose	Lignin	Pectic substances (as calcium pectate)
		Centimeters	*Number*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*	*Grams*
TOPS													
1	May 8	6–33	4,400	12.99	1.39	0.16	2.80	5.99	0.91	2.05	3.23	0.78	0.20
2	May 15	13–38	5,700	13.32	.98	.18	2.42	6.26	.82	2.34	3.54	.83	.20
3	May 22	15–38	4,100	20.27	1.54	.30	3.71	9.16	1.17	4.07	5.78	1.35	.27
4	May 29	19–53	3,300	30.49	2.21	.49	4.87	14.23	1.80	6.02	8.90	2.24	.42
5	June 5	21–62	2,400	41.33	2.80	.87	5.81	18.69	2.02	9.41	12.98	3.52	.36
6	June 12	26–71	1,800	57.37	3.08	1.27	6.13	28.59	3.01	12.42	17.44	4.86	.50
7	June 19	29–96	1,100										
8	June 23	63–112	350	101.57	6.79	2.45	10.44	48.20	4.85	23.27	33.42	8.18	1.02
ROOTS													
1	May 8		4,400	5.65	0.32	0.09	0.83	3.05	0.43	1.46	1.79	0.51	0.11
2	May 15		5,700	6.70	.29	.11	1.07	3.67	.47	2.04	1.90	.56	.11
3	May 22		4,100	8.50	.31	.14	1.41	4.60	.60	2.22	2.45	.68	.12
4	May 29		3,300	12.02	.37	.19	1.79	7.04	.71	2.83	3.21	.83	.14
5	June 5		2,400	14.75	.41	.23	2.28	9.02	.85	3.17	3.57	1.03	.16
6	June 12		1,800	19.25	.54	.25	2.33	12.07	1.08	3.76	4.56	1.35	.20
7	June 19		1,100	20.41	.64	.29	2.02	14.06	1.06	3.76	4.47	1.29	.16
8	June 23		350	24.92	.89	.42	2.81	16.00	1.36	5.39	6.25	1.99	.29

It has been suggested that lignin is formed by the plant from hemicelluloses (*21*), cellulose (*4*), and from pectic substances (*7*). The results presented in table 3 show definitely that lignin was not synthesized by the plant at the expense of either the pentosans, cellulose, or the pectic substances, as all these components increased as the plants grew and developed.

The pentosans, crude cellulose, lignin, and pectic substances in the roots showed a similar trend to those of the tops except, of course, that the increment in each case was considerably less.

DISCUSSION

Early in the development of many plants the cell walls undergo a change whereby the cellulose becomes hardened, or what is generally described as lignified. The change consists in the addition to the cellulose of a substance or a group of related substances designated as lignin. A knowledge of the quantity of lignin in a forage plant at different stages of its development or maturity and the possible precursors of lignin as well as the mechanism involved in its synthesis by the plant are of interest to the animal nutritionist. There is now considerable evidence to indicate that of all major plant constituents, lignin is one that is least readily digested by ruminants and nonruminants (*15*). A complete review of the various suggestions advanced concerning the nature of the parent substance and the possible mechanism involved in the synthesis of lignin by the plant was presented in a previous paper (*20*) from this laboratory and need not be repeated here in detail. Suffice it to state that among the precursors of lignin, the following have been suggested, namely, cellulose, hemicelluloses, and pectic substances. Table 3 shows that while the absolute quantity of lignin increased as the plant developed and matured, both the cellulose and the pectic substances also increased. There was no evidence whatever that lignin was synthesized by the plant at the expense of either the cellulose or the pectic substances. No direct determination of the hemicelluloses was made in this investigation for the reason that none of the available methods was found to be sufficiently precise or accurate for this purpose. However, the data on the percentages of furfural, particularly that derived from the pentoses of the polyuronides (table 2), may give indirectly an indication of the hemicelluloses present at various stages in the development of the plant. These results, it will be observed, do not support the claim that lignin is formed by the plant from hemicelluloses. The results are more in harmony with the suggestion made in a previous publication (*20*) that the plant synthesizes lignin directly from sucrose or from its hexose sugar components, namely, glucose or fructose. The possible mechanism involved in this transformation and synthesis was indicated in the paper already referred to (*20*).

SUMMARY

A study was made of the chemical composition of the tops and roots of the timothy plant at several stages of development. The quantities of the various constituents are presented on a percentage and on an absolute basis.

After an initial increase, the percentage of ash in the tops decreased as the plants grew older. The percentage of ash in the roots was rather irregular.

The percentage of nitrogen (and crude protein) in the tops decreased generally as the plants matured. The percentage of nitrogen (and crude protein) in the roots decreased in the early stages of growth of the plants and reached a minimum in the fifth harvest period, after which there was no significant change.

The quantity of nitrogen (and crude protein) in the tops increased rapidly as the plants developed and matured. The quantity in the roots showed little variation in the early stages of growth of the plants, but in the later stages the increase was somewhat more pronounced.

The percentage and absolute quantity of methoxyl in the tops increased regularly as the plants grew and developed. There was no appreciable variation in the percentage of methoxyl in the roots at successive stages in the development of the plants, although the quanquantity of methoxyl increased somewhat.

The quantities of the various extractives in the tops and roots increased steadily, although not uniformly, with the increase in the age of the plants.

The percentages of uronic acids in the tops and roots decreased generally, although not regularly, as the plants grew older. The absolute quantities of the uronic acids in the tops and roots generally increased as the plants developed and matured.

The percentage of total furfural in the tops generally showed an upward trend as the plants grew and developed. The percentage of total furfural in the roots was rather irregular.

The percentage of pentosans in the tops generally increased as the plants grew older. The percentage of pentosans in the roots was rather irregular, although the general trend was downward.

The percentage of crude cellulose in the tops generally increased, although not regularly, as the plants grew older. The percentage of crude cellulose in the roots was rather irregular.

The percentages of crude lignin and methoxyl in the lignin of the tops increased as the plant grew older. The percentage of crude lignin in the roots showed no significant variation, while the percentage of methoxyl in the crude lignin showed a slight trend upward as the plants grew older.

The percentages of pectic substances (as calcium pectate) in the tops and roots showed a general trend downward as the plants grew older.

The absolute quantities of the pentosans, crude cellulose, lignin, and pectic substances increased quite steadily as the plants grew older. There was no evidence of any kind to indicate that the plant synthesized lignin from either cellulose, pentosans, or pectic substances.

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STRUCTURE AND GERMINATION OF SEPTORIA SPORES [1]

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INTRODUCTION

An intimate knowledge of the structure of spores of pathogenic
fungi is of major importance to agricultural practice because of the
relation of spore structure to method of infection and possibly to the
effectiveness of control measures. Such knowledge has a definite
bearing on the development of effective chemical methods of prevent-
ing or controlling certain plant diseases. Current methods commonly
utilize spores of different fungi as test material in determining the
probable value of new and improved chemicals as fungicides The
mode of action and the effectiveness of specific substances as fungicides
depend in part upon the structure and the organization of the spores
with which they come in contact.

Septoria apii-graveolentis Dorogin, the cause of late blight of celery
(*Apium graveolens* L.), is widespread on cultivated and escaped celery
in California. The general appearance of the disease on cultivated
celery is somewhat different from that on escaped celery, but a study
of the fungus on the two types of host failed to reveal any significant
morphological differences. This study led to a further study of the
spores of this species of *Septoria*, with emphasis on the detailed struc-
ture of the spores, the meaning and nature of septation, the meaning
and nature of guttulae, the variation in the form of the spores, the
structural changes due to growth and germination, and the meaning
of maturity. The results of this study are reported herein. These
observations have a bearing on the structure of *Septoria* spores in
general, and this is briefly discussed.

MATERIAL AND METHODS

The spores of *Septoria apii-graveolentis* are about 30μ to 50μ long
and 1μ to 2.5μ in diameter. The spores have a highly elaborated and
detailed structure, which can be observed only at high magnifications.
The details of the structure cannot be delineated in the bright field
alone, and it is only by the use of the dark field under the most favor-
able circumstances that structure is revealed more completely. Ob-
servations made in the bright field alone are incomplete and sometimes
erroneous. The spores as seen under the microscope are too small to
photograph or to draw satisfactorily with the aid of the camera lucida.
The drawings reproduced herein represent the spores as actually seen

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and were prepared with precise attention to details of size, form, and relative position of the spore constituents. Projection at high magnification in a darkened room was a substantial aid in the work.

Celery leaves and leafstalks bearing pycnidia were selected in the field from either escaped or cultivated celery. Plants that showed reduction in vigor due to age or changes due to or symptoms of other diseases were never used. The leaves and leafstalks were put in the refrigerator at a temperature of 10° C. In a few hours spore horns (cirri) exuded from the pycnidia. Fresh spore horns provided the most uniform source of spores; they were free of extraneous matter and remarkably uniform in size and general condition. Unless spores from within the pycnidium were desired for special reasons, the spores of the spore horns were used throughout the study. After sufficient observations had been made to determine when *Septoria* spores are mature, only mature spores were used.

THE SPORE HORN, OR CIRRUS

The spore horn exudes from the ostiole of the pycnidium under some pressure as a threadlike structure, more or less curled. The amount of curling of the spore horn can be assigned to no specific cause, but it is believed to be due to conditions within the pycnidium and to the rapidity with which drying takes place as the horn emerges. The cross section of the horn is generally circular, but it may vary and conform somewhat to the shape of the ostiole. The length of the horns is variable. Some are only 2 to 5 mm. long, especially on thin leaves. Horns exuding from pycnidia embedded in the surface tissue of leafstalks are frequently 1 to 1.5 cm. long and occasionally as much as 2 cm. long. The color when freshly exuded is a light tan. The spores in the spore horn are held by some mucilage that is not seen. When a horn segment is placed in water the release of the spores from each other is immediate and complete, and no trace of any matrix or cementing substance can be observed. In the dry state the spores lie in the horn with their long axis parallel to that of the horn. The more slender or pointed end of the spore is the point of attachment within the pycnidium, and the spores are always oriented in the horn so that the distal or less pointed end emerges first. Although the ends of the spores are not markedly different in shape, there is ordinarily no difficulty in distinguishing the distal from the proximal end.

In an immature pycnidium the spores are bent and more crooked, sometimes with relatively abrupt bends, than they are later in the horns. This may be due to crowding, as the spores after being released from the horn and when floating free tend to become less bent or crooked. The spores usually have only a slight curvature, which is ordinarily apparent in only one plane, but the actual shape is more nearly that of a greatly stretched spiral.

STRUCTURE OF SPORES

The spore as observed in the bright field shows more or less conspicuous septations (p. 553) and guttulae (p. 554) as commonly described in the literature for nearly all species of *Septoria*. The spores stain readily for bright-field observation, with accentuation of the septations and some changes in the appearance of the guttulae. One of the most

suitable quick-acting stains is cotton blue. Dark-field observation was much more satisfactory in revealing the details of spore structure. The spores were commonly observed in the uninjured, unstained condition, wherein certain activities could be observed within the spore. The mature *Septoria* spore has an integument, which is regarded as the spore wall and which encloses four separate protoplasts, each surrounded by an exceedingly thin, flexible membrane. The spore is, therefore, four-celled. The integument is a thin, homogeneous membrane, without visible surface markings or structures. The inner surface is likewise smooth. It appears to be permeable, as stain and solutions pass through it readily and react with the protoplasts within but have little or no effect on the integument itself.

A number of methods were tried to stain the spore wall, including the techniques of Coleman (4)[2], Wisselingh (15) and others, as well as the methods used with chitin and hemicellulose. None gave satisfaction. The most successful stain was obtained by the following procedure. A clean segment of freshly exuded spore horn was placed in water on a clean glass slide, the spores were spread well, and the slide was dried very slowly and lightly in the flame, thus fixing the spores to the slide. The spot of spores was then covered with a 10-percent aqueous solution of potassium hydroxide, and the slide was warmed for a few minutes. The hydroxide was rinsed off with water, the spot was covered with a saturated aqueous solution of picric acid, and the slide was warmed again for a few minutes. The picric acid solution was rinsed off, and the stained spores were mounted in water under a cover glass. This treatment stained the wall a lemon-yellow color.

Plate 1, *A*, represents a mature unstained spore, as seen in the bright field. It shows the unequal density of the protoplasm of the spore and the true guttulae as spots or globules of variable size of light-colored or relatively transparent material. The alleged cross walls are distinct and appear to be continuous throughout with the outside enclosing wall. The outside enclosing wall has a substantial thickness, which is uniform over the length of the spore. The spore as represented shows that the bending is approximately at the points of septation; the reason for this will appear later (p. 551).

In the dark field of the microscope the normal unstained mature spore shows other characteristics (pl. 1, *B*). The spore wall is reduced to a thin line, and what appear to be cross walls in plate 1, *A*, have lost their identity. The guttulae no longer appear as well-defined spots. The four protoplasts may be distinguished from each other by the enclosing membranes of the separate protoplasts, but in the normal state their membranes are seldom conspicuous and are not seen in every spore. The contents of the protoplasts resolve into a colorless fluidlike medium in which there are small particles or granules shining in the reflected light. The particles tend to assemble in groups, usually separate from each other, the particles in a group not being agglutinated. A few particles are much larger than others, and the light reflected from the equatorial portion causes them to appear as rings. These may be some of the more darkly shaded portions of the bright-field drawing. Many of the bright particles of the protoplast are in fairly rapid motion, which is of the nature of

[2] Italic numbers in parentheses refer to Literature Cited, p. 559.

Spores of *Septoria apii-graveolentis:* A, Mature unstained spore as seen in the
bright field of the microscope; B, mature unstained spore as seen in the dark
field; C, mature spore plasmolyzed by heating and stained with basic fuchsin as
seen in the dark field; and D, ruptured, living, unstained spore in which ger-
mination has begun, as seen in the dark field.

·

Brownian movement, though other closely adjacent particles in the same group are relatively or wholly quiet.

Small particles are observed to rotate in a circle of small radius, and then suddenly to move in a more or less straight line to another part of the protoplast as much as a third or a half the length of the protoplast away, where the rotating motion begins again. The particle then returns to its former position and repeats the action indefinitely and with some regularity. There is no streaming movement of the protoplasmic contents. The grouping of the particles in the protoplast is such that there are often single spots as large as 15 to 25 percent of the volume of the protoplast that are free from particles, except as some may be moving across it. The groups of particles tend to settle toward the ends of the protoplasts, though a more general distribution is frequent. In no protoplast, stained or unstained, has there been observed any structure that could be construed as a nucleus. The areas within the protoplasts that are relatively free of particles permit the light of the bright field to pass through them with little interference and are the large guttulae so conspicuous in some species of *Septoria*.

The position, size, and distribution of the particles within the protoplasts in the dark-field view of the mature spore shown in plate 1, *B*, are characteristic of the unstained and uninjured spore. The points of septation are revealed very faintly at the abutment of the protoplasts at each end, but the point of septation does not show at the center. Though commonly absent, the cross member when present varies in appearance from spore to spore, depending on the position of the membranes relative to the source of illumination. When the protoplast particles are of sufficient size they are revealed as ringlike structures. Large particles that reflect wholly are either misshapen globules or material of a different sort.

The spores were treated with various solutions of sugar, salt, and other chemicals to induce plasmolysis, but without success. The cells appeared to be wholly permeable to such solutions. By carefully heating and drying the spores on a glass slide, the protoplasts can be shrunk so that they will draw away from each other and from the spore wall. Plate 1, *C*, represents a spore in which the contents were shrunk by heating and stained with basic fuchsin. The integument is revealed merely as a case that encloses the four protoplasts or cells. The protoplasts are detached from it, except as a mere matter of contact, and the integument appears to serve merely as a confining and protective covering. It is exceedingly thin—of the relative thickness illustrated in plate 1, *B* and *C*. The protoplasts have a membrane covering, which separates the contents of one cell from those of the next and also from the spore wall. The light reflected from the surface of the protoplast reveals a smooth, unmarred surface of no measurable thickness. The septa or cross walls that showed in the spore in the bright field have entirely vanished. In other words, there are no cross walls.

In the normal spore the protoplasts assume the shape of the integument and together completely fill it. Generally they abut at right angles to the spore wall, and so flexible is the membrane that the space is completely filled, as in plate 1, *B*. Nothing separates the protoplasts, and they lie end to end within the integument. The

transmitted light of the bright field passing through the area of contact of two protoplast membranes resolves itself into the septum or cross wall. The bending of the spore at the points of septation as shown in plate 1, *A*, is explained as follows: The pressure exerted by the turgidity of the protoplasts tends to stretch the integument. As the point of contact of the protoplasts, i. e., the so-called septum, is the most flexible in the spore, adjustments necessary in any spore under tension or pressure are best accommodated at these points. After the spore has been released from the confines of the pycnidium or horn its shape appears to depend on the integument. With the internal pressure relieved, as in plate 1, *C*, the tendency to curvature or bending is greatly lessened.

Plate 1, *D*, represents a spore, alive and beginning to germinate, that for some unknown reason has become ruptured. The integument has a definite break in the wall at one end. The protoplast adjacent to that end has also ruptured and collapsed, drawing the visible particle contents together within the shrunken membrane. The rest of the protoplasts, however, are alive, functioning normally, and growing. The live cell beyond the collapsed cell, released from the restraining pressure of the collapsed cell, has stretched out into the empty space without rupture or any apparent hindrance to its normal functioning. The single protoplasts seem to be units that function apart from the fate of the other units within the spore, though they commonly act in unison in germination and growth.

GROWTH AND GERMINATION OF SPORES

The *Septoria* spores from fresh celery material are not of uniform length, though at any single stage in their development they are relatively uniform in condition and aspect. The average length of 50 spores taken from a mature-appearing pycnidium examined within an hour after collection was 36.8μ. The spores were all three-septate and apparently wholly normal. An examination was made of 20 spores from a freshly exuded cirrus, and the same spores were followed through some successive germination stages. A suitable medium for the study of this process is celery juice, freshly extracted from petioles, filtered through paper, and used without sterilization. The slides were prepared by putting a segment of the cirrus in a drop of such juice, using a portion of the suspension as a hanging drop, and sealing and fastening the cover with rubber cement. The slides were held at room temperature, about 20° C. Instead of taking a large number of slides for observation, it was found best to use a single slide in which the conditions were uniform for all the spores under examination. As stated previously, the same 20 spores were observed throughout the period of observation, but they were not all the spores on the slide. Their average length when taken from the cirrus at the beginning of the period was 44.5μ. At the end of 4 hours their average length had increased to 50.4μ, with such a small corresponding increase in width that it could not be measured. In 5 hours the spores averaged 57.0μ in length, and in 6 hours they averaged 61.2μ.

During the next 2 hours there was no appreciable change in length, as the spores still averaged 61.2μ at the end of 8 hours. However, definite changes were then beginning. The spores were becoming

more crooked even at the end of 6 hours. Some were longer than others, but the shorter ones were swelling proportionately more than the longer ones. The swelling occurred over about one-half of the length. The contents of the spores seemed to be dividing into more protoplasts, as an increasing number of cells were apparent. At the end of 8 hours the increased diameter of the spores was very evident, bulges were appearing in many cells, and the protoplasts were more coarsely vacuolated than they were previously. At the end of 10 hours there were still more noticeable increases in diameters, and the swelling of the cells had become more pronounced. The protoplasts were separated slightly, and the central cells had become more heavily vacuolated. At this time the length averaged 62.3μ. During the next 2 hours growth was resumed, and at the end of that time the spores had reached an average length of 69.6μ. Branching was beginning, and it was evident that the spores had now germinated. The spore lengths of the single observation are summarized in table 1. Other series of observations on similar spore material gave like, though not identical, results.

TABLE 1.—*Length of spores of Septoria apii-graveolentis after different periods in celery juice*

Source	Number	Period	Length
		Hours	*μ*
Pycnidium	50	0	36 8
		0	44 5
		4	50 4
		5	57 0
Cirrus	20	6	61. 2
		8	61 2
		10	62. 3
		12	69 6

Another series of slides was prepared for germination study, with raw celery juice as a medium and with spores from a freshly exuded cirrus. Spores were selected for shorter length and were more nearly like those from a pycnidium. An attempt was made to promote germination as rapidly as possible. As the spores reached the end of the allotted periods they were flushed with a solution of cotton blue, which killed, fixed, and stained them and revealed the vacuolated structure in considerable detail. The spores were observed in the bright field of the microscope. The group (pl. 2) may be regarded as stages in the germination of a hypothetical *Septoria* spore, which may be considered as typical. The first spore, killed immediately, is shown in plate 2, *A*. The spore is 38μ long, slightly over 1.5μ wide at the widest point, and well filled with protoplasm staining to appear as finely divided, foamlike globules, some brighter than others. Brightness, however, may be a matter of position, as the larger particles do not appear to differ from the smaller except in size. The dissepiments appear as definite and substantial septations, which are continuous with the outside wall.

A second spore originally of the same size and aspect as that of plate 2, *A*, killed and stained with cotton blue at the end of 4 hours, is shown in plate 2, *B*. The spore has undergone several changes. The length has increased to 46μ and the width at the widest part to

A   B   C   D

SCALE OF MICRONS
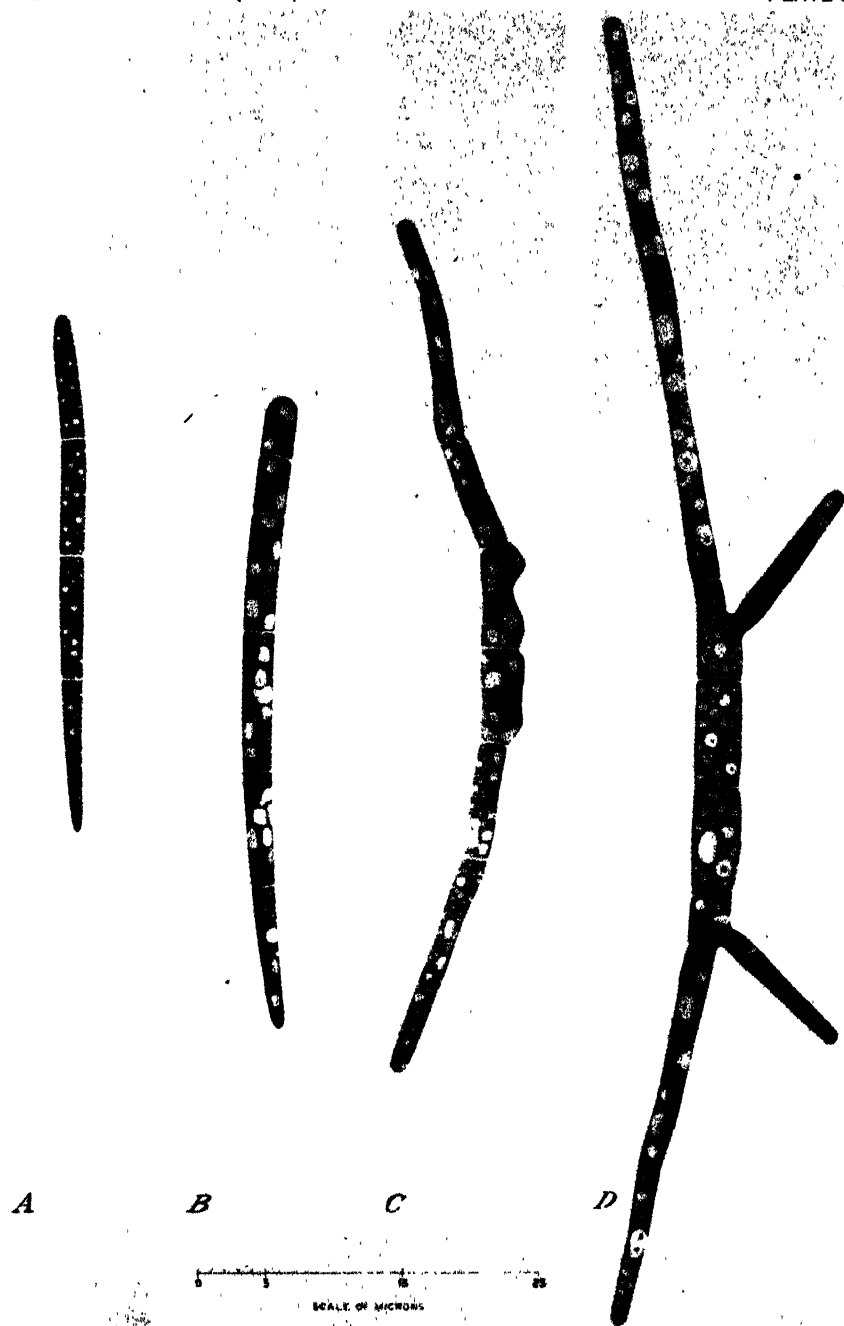
Stages in spore germination of *Septoria apii-graveolentis*. *A*, Mature spore from a freshly exuded cirrus. *B–D*, Germinating spores: *B*, at the end of 4 hours; *C*, at the end of 12 hours; *D*, at the end of 24 hours.

about 2.4μ.   The two end cells have divided so that the spore is five-septate, and the tapered end of the spore has increased in length more than the other cells.   The appearance of the spore contents has changed markedly from that of plate 2, *A*.   There are larger globules of uniform-appearing material, the finely divided structure is evident in the background, and the shape and size of the larger particles suggest that they are due to a flowing together or agglutination of the smaller particles to form the larger.   The contents appear to be similar throughout, and the undivided center cells show no distinction from the divided end cells.

Plate 2, *C*, represents a spore at the end of 12 hours.   The length has increased to 62μ, and the greatest diameter is nearly 3.3μ.   The two pairs of end cells of the type of spore shown in plate 2, *B*, have increased in length, and the ultimate cell on each end has divided again. The two center cells have shortened somewhat but have increased in diameter and apparently are under some pressure from within, as they are unequally bulged and extended.   The aspect of the contents is not markedly different from that of the spore in plate 2, *B*.   The dissepiments appear to be heavier and wider, and the several cells, except the two end ones, are becoming more crooked and unequally stretched.

The comparative spore development at the end of 24 hours is represented by plate 2, *D*.   The two center cells of the spore of plate 2, *C*, have here divided, and the outer cells of each division have sent out tubes similar in all respects to the growing end cells of plate 2, *C*. The center cells have grown slightly, the greatest diameter being about 3.75μ.   The length of this spore from tip to tip is 95μ, though a median line would be slightly longer.   The contents of the extended, slim ends are very highly elaborated, with larger guttulae than in the center cells.   The dissepiments now appear to be true cross walls, and not the membranes of contiguous protoplasts, as in the original spores. From this stage the germinating spore merges into mycelium and its identity becomes lost.   Under the conditions of the experiment the germination appeared to be retarded somewhat in the final stages, as though staleness of the medium, or byproducts, had checked the initial rate of growth.

MEANING OF APPLIED TERMS

In the routine examination of large numbers of *Septoria* spores from celery and other hosts, confusing variations have appeared in certain characters.   As species of *Septoria* have been defined in part in terms of septation and guttulae, these structures are of prime interest and should be understood more clearly.   Since the spore of the celery *Septoria* is characterized by certain features that apply throughout the genus *Septoria*, it is evident that the meaning of the terms applied to the spores needs to be evaluated.

SEPTATION

The spores of *Septoria* are commonly spoken of as "septate," but not always with a clear understanding of the implications of the word.   A great variety of structures, also designated by such names as "dividing wall," "membrane," "partition," and "dissepiment," have been called "septum."   Many of the authors of texts (as Owens, *11*) use the term

"cross walls," and in this they are justified because some mycologists—Ranojević (*13*), Petrak (*12*), Diedicke (*5*), and others—have used the term in their descriptions. In the case of *Septoria*, however, the term "cross wall" is incorrect since the connotation would justify the belief that the spores are chambered. "Septum" is not entirely free from this meaning, though it is subject to wide interpretation. The term "dissepiment," meaning a separating tissue, characterizes with some accuracy the structure that exists. Any term employed to mean a tissue, the function of which is to divide one part from another, is misused here. The tissues that separate the cells of the spore are the abutting plasma membranes of the cells; under natural circumstances these present the aspect of a single dissepiment. The term "septate," which is founded in the designation of the genus itself and cannot be abandoned, should be used, but with an understanding of the import of the word and its modification and application to special cases.

GUTTULA

The accepted meaning of "guttula" as a small drop-shaped spot, and "guttulate" as having drops or spots, is universally understood. The use of the term is very common throughout the literature of *Septoria*. A close study of a *Septoria* spore reveals some confusion as to the nature of the guttula, especially when it is realized that with many species this structure occupies an important part of the spore description. Stained, the spores reveal a great many guttulae, and these are correctly named in that they are small spots or droplets of protoplasmic origin or substance. But these are not the usual guttulae of the mycologists. The guttulae of the descriptive literature are bright spots caused by the transmitted light in the microscope passing through the clear areas of the cells of the spore, which, because of structural advantages, intensify the light as do small lenses. Such clear spots are shown in plate 1, *B*. The single cells may have a single bright spot, or they may have two, but the authors have observed no spores of any sort in which this feature was constant within a species as a single bright spot per cell. To base specific differences on this feature does not seem justified considering its nature and the likelihood that these spots are duplicated with equal variation in a great many species. To regard them as spots is justified, but to consider them as drops is not, and for that reason the term "guttulate" should be used with some reservation. To designate the places as "bright" or "lustrous" spots would be more nearly descriptive, though it does not seem to be possible to forego the use of the anomalous term "guttulate."

MATURITY

There seems not to be any special definition of the term "maturity" as applying to fungi, though in most cases it is assumed to mean that the fungus structure is capable of fulfilling its physiological function and can attain its functional destiny in the proper environment. This may apply to many fungus structures, such as pycnidia, perithecia, spores of all kinds, and even conidiophores and other parts. A mature fungus spore is a reproductive entity that has terminated physiological conjunction with the thallus that produced it and is capable of germination and growth in the proper environment.

The *Septoria apii-graveolentis* spore is borne on a conidiophore, first as a single-celled body; then with the formation of a single septum it becomes two-celled. The two cells divide simultaneously, so that the spore has three septa and four cells. When the spore attains three septa and four protoplasts or cells it has reached mature form, but it may or may not leave the pycnidium at this time. Until the spore is three-septate it is immature. Spores that have more than three septa have begun to grow and will germinate very soon unless growth is suspended. The interval between maturity and growth may be very short. Many spores appear to begin growth and then to suspend further activity. This is the reason that five- and seven-septate spores are sometimes found. This growth may occur while the spores are yet held in the cirrus, or a very few may be so found while in the pycnidium. There should be no spores with an even number of septa, as the manner of cell increase obviates them. The spore is mature when it becomes three-septate, and it is free to leave the pycnidium when environmental conditions permit.

A GENERAL CONSIDERATION[OF]SEPTORIA SPECIES

It is evident from the published descriptions of species of the genus *Septoria* as given by Saccardo *(14)* that the authors were generally uncertain regarding the septation of the spores. It is frequently impossible to compare specimens with published descriptions and to arrive at definite conclusions as to their identity, largely because of the different terms used in designating septation, or the wholly vague meaning of such terms. One finds such descriptive statements about spores as the following:

Term:	*Saccardo (14)*
Subcontinuis _ .	3: 481 (sp. 36).
Obsolete guttulatis .	3: 482 (sp. 37).
Obsoleteque septatis	3: 482 (sp. 42).
3–7 nucleolatis . _ .	3: 483 (sp. 48).
Obscure nucleolatis	3: 484 (sp 53).
Obscure septatis _ .	3: 485 (sp. 61)
Spurie 3–4-septatis.	3: 489 (sp. 83).
Pluri-nucleolatis. _	3: 491 (sp. 96).
Obsolete guttulato-septulatis	3: 495 (sp. 117).
Multiguttulatis . _ . _ .	3: 511 (sp. 212).
Indistincte nucleolatis	3: 518 (sp. 257).
Guttulatis vel septatis _ .	3: 519 (sp. 263).
Continuis v. plasmate obsolete partitis. .	25: 410 (sp. 25).

The situation in the genus *Septoria* has long been one of confusion. Beach *(2)* concluded that in certain species morphological characters vary considerably under different environmental conditions and that the value of measurements now given in specific descriptions is questionable. Garman and Stevens *(7)* made a comprehensive study of the descriptions of *Septoria* species based on those of Saccardo *(14, v. 1–22)*. They believed that spore length was an important clue to the proper placing of species. They summarized Saccardo's descriptions, giving, among other factors, the number of septa, number of guttulae, and spore shape for 1,181 species. To this summary of the statements on septation the authors have added a digest of 231 other species from Saccardo *(14, v. 25)* that have been classified in the manner of Garman and Stevens. This classification is shown in table 2.

TABLE 2.—*Supplemented summary of Garman and Stevens' classification of Saccardo's statements on septation in species of Septoria*

Citation	Classifier of statements	Species	Statements about septation			
			Absent	Indefinite	Definite	
					Septa present	Septa absent (spores continuous)
		Number	*Percent*	*Percent*	*Percent*	*Percent*
Saccardo (*14, v. 1-22*)	Garman and Stevens (7)	1,181	36.5	17 4	28.5	17.7
Saccardo (*14, v. 25*)	Authors	231	21.2	14.7	40.7	23.4
Total		1,412	34 0	16.9	30.5	18 6

Table 2 represents at best only an incomplete analysis of the septation statements found in the descriptions of *Septoria* as given by Saccardo (*14*). Approximately 34 percent of the descriptions have no reference to septation whatever, and 18.6 percent of the species are said to have continuous spores (septa absent); from these last no inferences are justified. Approximately 16.9 percent have indefinite statements about septation. Of the 30.5 percent that are described in terms of septation, a further analysis reveals that in the majority of cases the specifications are vague and possibly misleading, and nothing conclusive is warranted by further segregations. It may be doubted whether many of the species with one-septate and even-number-septate spores belong to *Septoria* at all, if the observations recorded herein are correct. Beach (*2*) found that septation appeared in some of the species with which he worked, when they were properly stained, but he did not always state how many septa were revealed. Hemmi (*9*), in describing *S. glycines*, found the spores to consist of "one to three, rarely four cells," which would indicate that they were continuous, one- or two-, and rarely three-septate, but that the septa might be easily overlooked. On germination the septa showed clearly. Wolf and Lehman (*16*), describing *S. glycines* Hemmi, illustrated one-, two-, and three-septate and continuous spores. Frank (*6*), studying *S. avenae*, found the spores to be two- to four-septate. The illustrations of *S. sisymbrii* given by Ranojević (*13*) show one-, two-, three-, four-, and five-septate spores, a condition that is untenable in the authors' understanding of the development of mature *Septoria* spores.

The description given by Saccardo (*14, v. 25, p. 454*) for *Septoria apii-graveolentis* contains no mention of spores whatever. Cochran (*3*) studied these spores with great care and found that the number of septa varied from zero to seven. The spores studied for this character were classified by Cochran as follows:

Number of septa per spore: *Percent of spores*

     Less than 3 _____ 10

     3 _____ 60

     4 _____ 18

     5 _____ 10

     More than 5 _____ 2

The 10 percent of the spores that had less than three septa undoubtedly were immature and may have consisted largely of one-septate spores, though what appeared to have been two-septate spores may have been observed. The three- and five-septate spores would be normal, especially as germination had evidently begun as shown by the presence of the five-septate and more-than-five-septate spores. The authors, however, cannot account for such a large proportion as 18 percent of four-septate spores.

DISCUSSION

The authors have examined a number of *Septoria* species from widely varied hosts and find that the odd-number-septate spores are characteristic throughout. The spores from *Septoria* species producing cirri are generally of larger size and may have a higher number of septa, as five and seven, and possibly more. Three is the number most commonly found. A few of the many species examined were *Septoria dulcamarae* Desm. on *Solanum dulcamara* L.; *S. solitaria* Ell and Ev. on *Rhododendron occidentale* Gray; *S. silenicola* Ell. and Mar. on *Silene gallica* L.; *S. alnifolia* Ell. and Ev. on *Alnus rhombifolia* Nutt. and on *A. rubra* Bong.; *S. scabiosicola* Desm. on *Scabiosa arvensis* L.; *S. aceris-macrophylli* Pk. on *Acer circinatum* Pursh; *S. angularis* Pearn. and Barth. on *Solidago latifolia* L.; *S. populi* Desm. on *Populus trichocarpa* Torr. and Gray; *S. rubi* Westd. on *Rubus vitifolius* Cham. and Schlecht.; *S. pentstemonicola* Ell. and Ev. on *Penstemon cordifolius* Benth.; *S. stachydis* Rob. and Desm. on *Stachys californica* Benth.; *S. scrophulariae* Pk. on *Scrophularia californica* Cham. and Schlecht.; and *S. corylina* Pk. on *Corylus californica* (A. DC.) Rose. *Septoria* on celery plant parts and seeds from many parts of the world was examined.

The examination of a number of fungi now known as species of *Septoria* indicates that those characterized by shorter and wider spores and with one or two septa are probably misclassified in many instances and should be critically examined for possible transfer to some other genus. Some of the species examined would more properly find their places in *Ascochyta*, *Diplodina*, *Hendersonia*, or elsewhere; and among the many-septate forms are those that may belong in *Stagonospora*, *Rhabdospora*, and possibly *Cylindrosporium*. As Garman and Stevens (7), Beach (2), and others have pointed out, the greatest confusion exists in the genus *Septoria* and the entire genus is in need of study and revision. As a mycological study, Grove's (8) treatment of the species of *Septoria* is the most adequate one so far. Diedicke (5) found cause to reclassify many species of *Septoria*, and a detailed study of the pycnidial formation and structure would be required to establish the proper relation of these species, as pointed out by Archer (1) in the case of other Sphaeropsidales.

The manner of septation in *Septoria apii-graveolentis* as the authors have found it and the uncertainty that results from observing these minute spores solely by means of the bright field of the microscope indicate that observation by the dark-field method is necessary for a reliable estimate of the number and kind of dissepiments in these spores. The customary manner of spore septation as it appears in

S. apii-graveolentis, by simultaneous division of the end cells after the formation of the primary septum, indicates that an even number of septa in these spores is anomalous. Though even-number-septate mature spores are commonly reported to be present, the authors have never observed one in this species. Other species also show the odd-number-septate structure consistently, though the dark field of the microscope usually has to be resorted to to make the odd-number septation distinguishable.

The spores of fungi within a species are generally very uniform in size, structure, and other characters, varying only in small percentages from the normal. Ranojević (*13*), however, credited *Septoria sisymbrii* with spores varying in length from 19μ to 62μ. Leonian (*10*) found that a higher food concentration in culture produces more numerous pycnidia and that rich hyphal growth and pycnidial development are parallel within a very wide range. From the authors' study of the celery *Septoria* it seems certain that the spores become mature on attaining the three-septate condition, but not before. It is possible that the phenomenon of suspended germination is common throughout the genus *Septoria* and forms the basis for the wide variation reported in the size and septation of the spores, multiseptation especially being an evidence of growth after maturity. From mycologists greater detail should be required in the definition of the conditions under which the fungus was found and the environment to which it was exposed. As no rest period is necessary, complete germination may occur promptly. This is evidently a factor in the spread of the disease caused by this fungus.

CONCLUSIONS AND SUMMARY

A detailed study has been made of the spores of *Septoria apii-graveolentis* Dorogin from cultivated and escaped celery. The structure of the spore and its growth and germination are given extended treatment. The meaning and significance of terms applied to the spore, such as "septation," "guttula," and "maturity," are analyzed.

Assuming that the spores of *S. apii-graveolentis* reveal the nature of *Septoria* spores in general, the writers have discussed the inadequacy and confusion existing in the descriptions of species in the genus *Septoria* and have offered suggestions for greater precision in description and care in observation.

The following conclusions have been reached:

(1) The spores of *Septoria apii-graveolentis* are mature when they consist of four cells, i. e., when they are three-septate.

(2) Septation in the spores of this fungus is caused by the abutting membranes of contiguous cells within the integument. There is no wall or true septum within the mature spore.

(3) The guttulae of the *Septoria* spores in descriptive mycological literature are bright spots, produced by lens action on the clear portions within the cells of the spore.

(4) Germination is accompanied by cell proliferation, usually by division of the end cells of the spore. There are changes in the appearance of the cell contents with progressive stages of germination.

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EXPERIMENTS WITH ARSENITE SPRAYS TO ERADICATE SCLEROTINIA LAXA IN STONE-FRUIT TREES AS A MEANS OF CONTROLLING THE BROWN ROT DISEASE IN BLOSSOMS [1]

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INTRODUCTION

The development of eradicant fungicides has received increased attention since Keitt and Palmiter (15) [2] described experiments conducted over a 13-year period (8,9,10,14,16, 17,18,21). According to these workers, the survival level of a fungus within the orchard is lowered by sprays applied at a vulnerable stage of its development. Thus the ascigerous stage of *Venturia inaequalis* proved vulnerable to a copper-lime-arsenite mixture applied to the leaves before they fell or after they were on the ground. The copper-lime-arsenite mixture was prepared by combining copper sulfate, lime, and monocalcium arsenite. In preliminary tests with other fungi, these writers found that this fungicide destroys such diverse parts as mycelia, conidia, pycnidia, exposed and embedded ascocarps, sclerotia, and basidiospores. Tims (25) furnishes additional evidence that it is effective against sclerotia of *Corticium stevensii* and *C. microsclerotia* on fig trees.

In 1939 Keitt (11) reported successfully using "sodium dinitro-o-cresol" (Elgetol) against the perithecial stage of *Venturia inaequalis*. Further evidence of its effectiveness against this fungus is reported by Keitt, Clayton, and Langford (12), Keitt, Clayton, and Moore (13), Palmiter (20), Sharvelle (23), and Kadow and Hopperstead (7). Anderson (1) found that it prevented ascospore development by *Coccomyces hiemalis* in sour cherry leaves, and Sharvelle (23) used it against *Plectodiscella veneta* on raspberry canes. Strong and Cation (24) report that "Elgetol regular" in 1-percent strength prevented the extension of the telial columns and killed teliospores of *Gymnosporangium globosum*, *G. juniperi-virginianae*, *G. claripes*, and *G. clavariaeforme* in the galls on *Juniperus virginiana* and *J. communis*.

Various other materials apparently have some value as eradicants. In the small-scale trials of Holz (4), unoiled calcium cyanamide applied dry to apple leaves lying on soil prevented perithecial development of *Venturia inaequalis*. By dusting the orchard floor with this material, Huber and Baur (5) and Huber, Baur, and Breakey (6) destroyed apothecia of *Sclerotinia fructicola*. Hendrick (2) determined that urea, ammonium sulfate, ammonium chloride, ammonium carbonate, potassium sulfate, potassium chloride, and lime-sulfur had deleterious effects on the ascigerous stage of *V. inaequalis*. The successful use of paradichlorobenzene against the tobacco downy mildew fungus [3] by

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McLean and Pinckard (*19*) and by Pinckard (*22*) shows how the gaseous phase of a material may kill the fungus in living tissue without unduly injuring the host.

In 1938 and 1940 the writer and a coworker (*26, 27*) published the results of tests with calcium, zinc, and sodium arsenites, applied to apricot and almond trees to suppress the sporodochia of *Sclerotinia laxa* on blighted hold-over twigs. The present paper gives the details of these trials, together with results secured in 1940.

Certain other materials, such as Elgetol, paris green, and tar oil, were tested; but as further trials with these materials are necessary before conclusions can be drawn, the results are not reported here.

THE BROWN-ROT DISEASE OF STONE FRUITS

The California stone-fruit industry suffers severe losses from blossom and fruit infection caused by two species of *Sclerotinia*. Upon finding apothecia of *Sclerotinia fructicola* (Wint.) Rehm to be fairly abundant on peach mummies on the orchard floor, Hewitt and Leach (*3*) studied the distribution of this species and of *S. laxa* Aderh. and Ruhl. They determined that whereas *S. fructicola* was primarily responsible for peach-fruit rot, *S. laxa* was almost exclusively responsible for serious blossom blight in apricots and almonds.

Since none of the apothecia that Hewitt and Leach found were those of *Sclerotinia laxa*, their studies show, like those of others, that the perfect stage of this species if produced at all is exceedingly rare. The fungus (*S. laxa*) causing blossom infection in apricot and almonds overwinters in the twigs that are blighted after blossom infection, and (in apricot) on the occasional rotted fruit that hangs on the tree throughout the winter. Apparently, therefore, the cycle of development of *S. laxa* is confined to the trees. The primary inoculum for blossom infection is conidia produced on sporodochia, the spore-bearing cushions (fig. 1) which develop on twigs and mummied fruits during the winter. As fruit rotting by this fungus is comparatively uncommon in apricot and apparently unknown in almond, the major source of conidia is blighted twigs.

DEVELOPMENT OF SPORODOCHIA IN RELATION TO SEASON

After blossom infection in the spring, *Sclerotinia laxa* enters the small twigs and blights the portions distal to the point of entry. Conidia are produced on blighted blossom parts during early summer, but are less abundant in the late summer and autumn, and probably do not survive the winter. Sometime during the winter the sporodochia develop on the blighted twigs and blossoms, and immediately produce conidia (fig. 1). Observations on the period of winter during which the sporodochia develop are given below.

To judge from casual examinations made during the winter of 1937–38, sporodochial development began sometime in early February, none having been observed on almond trees in late January. During the winter of 1938–39, when more systematic observations were made, sporodochia were found on both almond and apricot twigs in late December. As figure 2 indicates, they became numerous on almond twigs between about December 27 and February 7, after which few or none appeared. In a prune orchard, on the other hand, they began to appear somewhat later and increased in number more slowly than in the almond orchard.

FIGURE 1.—Sporodochia (arrows) produced by *Sclerotinia laxa* on almond twigs
in 1938. These structures, produced during the winter, bear conidia which
are the only source of blossom infection the following spring. It is to prevent
the development of these sporodochia, or to kill them (and the conidia thereon)
after they develop, that eradicant sprays are applied in winter.
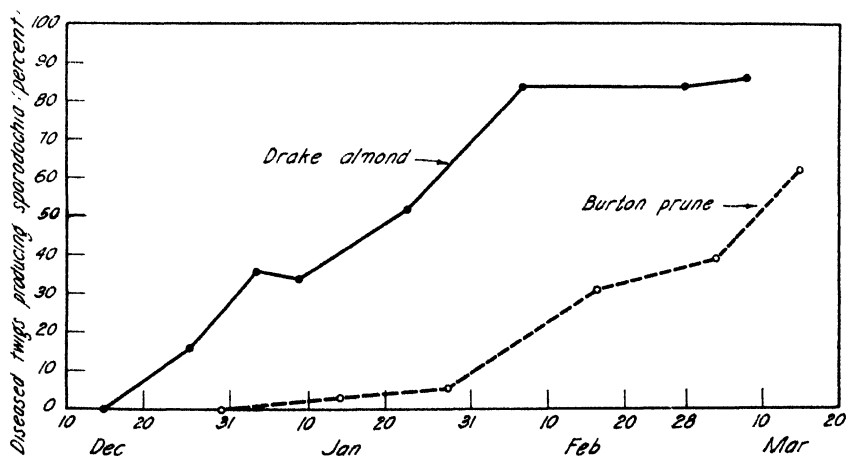
FIGURE 2.—Seasonal development of sporodochia during the winter of 1938–39
on blighted hold-over twigs in trees of Drake almond and Burton prune, as
expressed by the percentage of blighted twigs bearing visible sporodochia at
each succeeding date of examination.

Variations in the time sporodochia developed in different districts were marked in 1938–39. In the central valleys (Sacramento and San Joaquin) they developed on almonds and apricots over about the same period as indicated for almond in figure 2, but in coastal districts (Santa Clara and Pajaro Valleys) they appeared later.

In the winter of 1939–40 these structures did not begin to appear until late January and were not abundant until after the middle of February. As in 1938–39, they developed later in coastal counties than in the interior.

PRESENT CONTROL METHODS

Present measures for controlling the serious blossom blight of apricots and almonds consist in applying bordeaux mixture to the blossoms as they emerge from the bud scales in the spring To cover the rapidly expanding blossoms completely and to insure successful control, two sprays are frequently necessary—one immediately after blossoms emerge, and another when some of the blossoms are open. Reducing the hold-over sources of the fungus, by removing blighted twigs and mummied fruit from the tree, is recognized as a desirable supplementary procedure.

When conditions strongly favor development of the disease, as during the past 4 years, successful control is difficult for the following reasons: (1) Frequent rains in the spring may prevent application of the fungicide at the critical period; (2) the critical period in certain years is too short to permit coverage of large orchards; and (3) removal of all blighted twigs and mummied fruits from the trees is impractical and too expensive.

ELIMINATING THE SPORODOCHIAL STAGE WITH ERADICANT SPRAYS AS A MEANS OF CONTROLLING THE BROWN ROT DISEASE IN BLOSSOMS

The presence in the trees of the only known hold-over stage of *Sclerotinia laxa* would seem to afford an opportunity for attacking the fungus at a vulnerable point. Could this stage be eliminated or greatly reduced, the incidence of the disease should be lessened. The relatively inefficient method of removing blighted twigs and mummied fruits has, in certain instances, reduced the blossom blight; but in case of epidemic development the blighted twigs are too numerous to be removed. A spray capable of eliminating the sporodochial stage is, therefore, desirable. Whether it prevents development of the sporodochia or destroys them (and the conidia borne thereon) after they develop depends, of course, upon its properties and upon the time of application in relation to their initiation. In any event, the period of application would be sometime during the dormancy of the trees.

EFFECT OF ARSENITE SPRAYS ON THE DEVELOPMENT OF SPORODOCHIA

According to the observations reported earlier (fig. 2), the period of maximum sporodochial development, though varying somewhat from year to year and from district to district, extends from early January until late February. If, therefore, sprays should be applied before mid-January, or in some years before early February, few sporodochia would be present. To be of value at this time the spray

should be capable of preventing the development of sporodochia as well as killing the few already present.

This section reports the results of spraying with calcium, zinc, and sodium arsenites before sporodochia developed in large numbers. The effect of these materials on the fungus was gauged by determining the abundance of sporodochia on 100 to 150 blighted hold-over twigs, collected at random from each sprayed and unsprayed plot during the blossoming period or shortly after. In certain orchards where sporodochial development was followed closely, 25 twigs from each tree in the plot were examined. These examinations were repeated several times on some of the more important plots. To observe the sporodochia the twigs were held in a strong light under a hand lens.

According to observations made early in the work, the effect of the sprays on sporodochial development can be shown (1) by ascertaining the percentage of blighted hold-over twigs that bear sporodochia, and (2) by determining, on twigs that bear sporodochia, the average number of these structures per twig. As neither of these values alone gives the entire picture of sporodochial abundance, both are presented in the tables, together with their product as an index number. This index number should be regarded as evaluating the frequency of sporodochia on the samples collected and not as measuring the total present in the trees. To obtain a measure of the total present, one would have to determine the total number of hold-over twigs, a task for which no feasible procedure has been devised.

SPRAY MATERIALS AND METHODS OF APPLICATION

The monocalcium arsenite used (hereafter designated as calcium arsenite) was a finely ground powder containing 69 percent of arsenic as As_2O_3 and 22 per cent of As_2O_3 in water-soluble form.

The zinc arsenite (so-called buffered) was a finely ground powder containing 35 percent of As_2O_3 and 0.5 percent of water-soluble arsenic.

Solutions of sodium arsenite sold as weed killers were employed in most cases. The solutions were used at a rate which would be equivalent to 1, 2, or 3 pounds of sodium arsenite per 100 gallons of water.

Bordeaux mixture added to calcium and zinc arsenites was prepared from finely ground copper sulfate and hydrated lime. The calcium or zinc arsenite was added to the spray tank after the bordeaux was prepared.

The zinc sulfate-lime combination used with calcium arsenite in a few tests was prepared by dissolving the zinc sulfate in water in the spray tank and adding hydrated lime suspended in a small amount of water. The calcium arsenite was then added.

The oil added to the arsenites was a dormant type of petroleum emulsion having a 70 percent unsulfonated residue.

Other supplemental materials were potassium ethyl xanthate in powder form and Hydrolene No. 66, a powdered organic wetting agent. The procedure by which the latter material was combined with calcium arsenite in one test will be described at the proper place.

The sprays were applied as a fine mist to all twigs and branches, care being taken to avoid drenching. Except where noted, the spray dried thoroughly before rains occurred.

COMPARISON OF THE EFFECTS OF DIFFERENT CONCENTRATIONS OF CALCIUM ARSENITE

Five Drake almond trees sprayed on January 25, 1938, with calcium arsenite, 4 pounds per 100 gallons, were examined on February 17. Between these dates numerous sporodochia developed in unsprayed trees, but few developed in the sprayed trees (table 1). Later examinations, moreover, revealed no further development of these structures in sprayed trees, though a measurable increase occurred in unsprayed trees.

The following winter, 1938–39, 1, 2, and 4 pounds of calcium arsenite per 100 gallons of water were applied in a number of apricot and almond orchards and in one prune orchard (tables 2 and 3 and fig. 3).

TABLE 1.— *Development of sporodochia and of blossom blight in almond trees sprayed with calcium arsenite, zinc arsenite, alone and with oil, and sodium arsenite, 1938*

Treatment [1] (Pounds per 100 gallons)	Trees observed	Twigs with sporodochia (A)	Average number of sporodochia per twig [2] (B)	Index number (A×B)	Blossoming twigs blighted [3]
	Number	*Percent*			*Percent*
Unsprayed	7	69	5 4	373	62
Calcium arsenite 4-100	5	12	1 4	17	14
Calcium arsenite 4-100+4 percent oil	5	3	1 0	3	13
Zinc arsenite 4-100	1	22	2 1	46	30
Zinc arsenite 4-100+4 percent oil	3	35	2 1	74	27
Sodium arsenite 1 2 100	2	50	5 0	250	52

[1] Sprays were applied Jan 25 before sporodochia developed
[2] Observations on sporodochial development were made Feb 17 These values were obtained by dividing the total number of sporodochia on the samples by the number of twigs which produced sporodochia
[3] Disease data were taken after twigs which bore infected blossoms were blighted

TABLE 2.— *Development of sporodochia and of blossom blight in apricot and in almond trees in different orchards sprayed at different dates with three concentrations of calcium arsenite used alone and in combination with other materials, December 1938 and January 1939*

APRICOT

Orchard No [1]	Date sprayed	Treatment (pounds per 100 gallons)	Twigs with sporodochia (A)	Average number of sporodochia per twig (B)	Index number (A×B)	Blossoming twigs blighted
			Percent			*Percent*
		Unsprayed	79	9 8	774	29. 0
		Calcium arsenite 4-100	2	1 0	2	3
		Calcium arsenite 4-100+4 percent oil	3	1 0	3	1
		Calcium arsenite 4-100+2 percent oil	5	1 0	5	. 2
		Calcium arsenite 4-100+bordeaux mixture 8-4-100	4	1. 0	4	. 1
11	Dec 5-8	Calcium arsenite 2-100	7	2 7	19	. 2
		Calcium arsenite 2-100+2 percent oil	3	1.3	4	. 5
		Calcium arsenite 2-100+bordeaux mixture 8-4-100	4	2. 0	8	. 2
		Calcium arsenite 1-100	16	2. 6	42	. 2
		Calcium arsenite 1-100+2 percent oil	12	2. 4	29	. 2

[1] Locations of the orchards were as follows 11 and 11A, Contra Costa County; 14, Sacramento County; 15, Monterey County.

TABLE 2.—*Development of sporodochia and of blossom blight in apricot and in almond trees in different orchards sprayed at different dates with three concentrations of calcium arsenite used alone and in combination with other materials, December 1938 and January 1939*—Continued

APRICOT—Continued

Orchard No.	Date sprayed	Treatment (pounds per 100 gallons)	Twigs with sporodochia (A)	Average number of sporodochia per twig (B)	Index number (A×B)	Blossoming twigs blighted
			Percent			*Percent*
		Calcium arsenite 1-100+bordeaux mixture 8-4-100.	17	2.1	36	0 3
		Calcium arsenite 4-100	3	2.3	7	3
		Calcium arsenite 4-100+2 percent oil	2	1 0	2	.2
		Calcium arsenite 4-100+bordeaux mixture 8-4-100	7	1.1	8	.1
		Calcium arsenite 2-100	6	1 2	7	.1
		Calcium arsenite 2-100+2 percent oil	3	1.3	4	.2
		Calcium arsenite 2-100+2 percent oil (120-tree plot) [2]	4	1.1	4	.4
11	Jan 18-19	Calcium arsenite 2-100+bordeaux mixture 8-4-100	6	1.7	10	.3
		Calcium arsenite 2-100+bordeaux mixture 8-4-100 (120-tree plot) [2]	9	1 9	17	.6
		Calcium arsenite 1-100	7	4.0	28	1.2
		Calcium arsenite 1-100+2 percent oil.	7	2.4	17	.3
		Calcium arsenite 1-100+bordeaux mixture 8-4-100.	16	2 0	32	3 0
		Unsprayed	68	6 9	469	45
	Dec 27	Calcium arsenite 4-100	3	1 3	4	2
		Calcium arsenite 4-100+2 percent oil	3	1 4	4	2
11A		Calcium arsenite 4-100+bordeaux mixture 8-4-100	5	1 3	7	2
	Jan 18	Calcium arsenite 4-100+1 percent oil	2	1 5	3	3
		Calcium arsenite 4-100+bordeaux mixture 8-4-100.	4	1 6	6	3
		Unsprayed	79	10 1	798	-----
	Dec 15 16	Calcium arsenite 4-100	4	1 0	4	-----
		Calcium arsenite 4-100 +2 percent oil	1	1 0	1	-----
		Calcium arsenite 2-100	13	3 3	43	-----
15		Calcium arsenite 2-100+2 percent oil	15	3 1	47	-----
		Calcium arsenite 1-100	39	3 3	129	--
		Calcium arsenite 4-100	7	1 0	7	--
	Jan 30 31	Calcium arsenite 4-100+4 percent oil	2	1 5	3	--
		Calcium arsenite 2 100	5	1 4	7	-----
		Calcium arsenite 2 100+4 percent oil	5	2 1	11	-----
		Calcium arsenite 1-100	11	2.1	23	-----

ALMOND

		Unsprayed	85	6 8	578	33
		Calcium arsenite 4-100	2	1 0	2	3
		Calcium arsenite 4-100+2 percent oil	3	2 4	7	3
		Calcium arsenite 4-100+bordeaux mixture 8-4-100	4	3 3	13	8
	Dec 13	Calcium arsenite 2-100	1	1 1	1	4
		Calcium arsenite 2-100+2 percent oil	4	1 1	4	7
		Calcium arsenite 2-100+bordeaux mixture 8-4-100.	22	3.1	68	20
14____		Calcium arsenite 1-100	18	2 9	52	9
		Calcium arsenite 1-100+2 percent oil.	15	3.1	47	10
		Calcium arsenite 1-100+bordeaux mixture 8-4-100	63	3 3	208	35
		Calcium arsenite 4-100+bordeaux mixture 8-4-100.	4	1 5	6	3
		Calcium arsenite 2-100	7	1.9	13	6
	Jan. 17____	Calcium arsenite 2-100+2 percent oil	3	1.0	3	4
		Calcium arsenite 2-100+bordeaux mixture 8-4-100	17	2 9	49	6
		Calcium arsenite 1-100+2 percent oil__	17	2.2	37	11

[2] The 120-tree plots were sprayed with a large spray rig operating at 400 to 500 pounds pressure. Other plots were sprayed with a small rig operating at 275 pounds' pressure

TABLE 3.—*Development of sporodochia and of blossom blight in apricot and in prune trees in different orchards sprayed at different dates with four concentrations of calcium arsenite, used alone and in combination with other materials, 1938-39*

County	Orchard No. and kind of trees	Treatment (pounds per 100 gallons) and date sprayed	Twigs with sporodochia (A)	Average number of sporodochia per twig (B)	Index number (A×B)	Blossoming twigs blighted
			Percent			*Percent*
Tehama	1; apricot	Unsprayed	49	4 9	240	39
		Calcium arsenite 4-100, Dec. 29	12	3.5	41	17
		Calcium arsenite 4-100+2 percent oil, Dec. 29.	8	2.7	22	10
Stanislaus	5, apricot	Unsprayed	70	9.4	658	46
		Calcium arsenite 2-100+2 percent oil, Jan. 20.	13	2.8	36	16
	12, apricot	Unsprayed	45	4 8	216	72
		Calcium arsenite 4-100+2 percent oil, Jan. 19.	1	1.0	1	65
		Calcium arsenite 2-100+2 percent oil, Jan. 19.	5	1.0	5	74
San Joaquin	9; apricot	Unsprayed	49	3 9	191	76
		Calcium arsenite 4-100, Nov. 30	3	1 0	3	49
		Calcium arsenite 4-100+bordeaux mixture 10-10-100, Nov. 30.	18	1 6	29	71
		Calcium arsenite 4-100, Nov. 30; sodium arsenite 1-100, Feb. 17.	....	.... ..	.....	40
		Calcium arsenite 4-100, Jan 19	4	1.5	6	38
Yolo	13; prune	Unsprayed	68	4 7	320	... ...
		Calcium arsenite 4-100+2 percent oil, Dec. 12.	6	1 2	7	....

That sporodochia developed more numerously in some orchards than in others is seen from variations in the index numbers of unsprayed plots. Unlike the 1940 results to be reviewed later, the efficacy of a given treatment did not appear to be influenced by the intensity of sporodochial development. Calcium arsenite 4-100, for example, was applied in 8 orchards and calcium arsenite 4-100 plus oil emulsion in 7. The sporodochial index numbers of untreated plots varied from a low of 191 to a high of 798, yet these treatments were no less effective at the higher than at the lower levels. Comparisons of the 1939 results secured in different orchards, therefore, do not require that the indices of treated plots be referred to the unsprayed indices, unless the percentage reduction in sporodochial numbers is desired.

At the rate of 4 pounds per 100 gallons of water, calcium arsenite was highly efficient in reducing sporodochial development; rates of 2-100 and 1-100 were less efficient. As compared with the check, however, the 1-100 concentration when used alone reduced these structures on an average 92 percent; the 2-100 concentration 97 percent. Between orchards, however, the 1-100 concentration varied more in effectiveness than the 4-100 concentration; obviously it was too weak for consistent results. The 2-100 concentration was decidedly more efficient; and since, as noted later, it injured the trees less than the 4-100, it seemed a promising strength for further trials.

Attention was paid particularly to the effect of calcium arsenite on the development of sporodochia in the cankers that formed on large limbs of almonds. Because of the thick and roughened periderm on large limbs, the suppression of sporodochia in cankers formed thereon

is presumably more difficult than on smaller twigs. Concentrations of 2–100 and above appeared, however, to prevent sporodochial development on these cankers as effectively as on small twigs.

On the basis of the 1939 results, 2 to 4 pounds of calcium arsenite per 100 gallons of water appeared to be the most promising concentrations. Because of injury by the latter strength, most of the 1940 treatments were given with 2 and 3 pounds per 100 gallons. Almonds had proved so sensitive to arsenites that concentrations greater than 2–100 were not used in 1940.
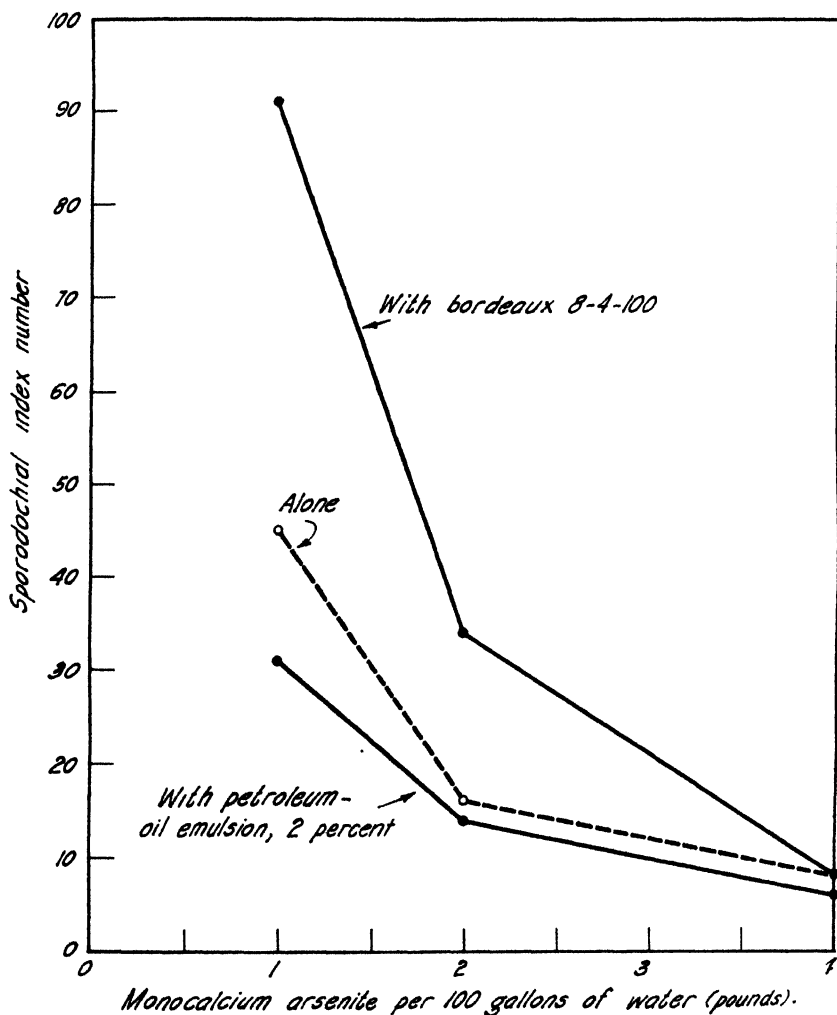
FIGURE 3.—Effect on sporodochial development of three concentrations of calcium arsenite used alone and in combination with petroleum-oil-emulsion and bordeaux mixture. As the sporodochial index numbers were obtained by multiplying the percentage of hold-over twigs with sporodochia by the average number of sporodochia per twig, the higher the index the lower is the efficacy of the spray.

In 1940 four varieties of plums—Wickson, Formosa, Sharkey, and Kelsey—were sprayed with calcium arsenite at the rate of 2–100 and 1–100; but because of insufficient holdover sources, results on sporodochial suppression were obtained only with Wickson.

Development of sporodochia began so late in the winter of 1939–40 that practically all applications were made before these structures developed in numbers. The details regarding dates of spraying, together with the results on sporodochial suppression, appear in tables 4 and 5. Before discussing the results, however, a word is necessary about the effect of the sporodochial level on efficiency of supression.

TABLE 4.— *Development of sporodochia and of blossom blight in apricot and in plum trees in different orchards sprayed at different dates with various concentrations of calcium arsenite used alone and in combination with other materials, 1940*

County	Orchard No. and kind of trees	Treatment (pounds per 100 gallons) and date sprayed	Twigs with sporodochia (A)	Average number of sporodochia per twig (B)	Index number (A×B)	Blossoming shoots blighted
			Percent			*Percent*
	1; apricot	Unsprayed	51	6 8	347	39
		Calcium arsenite 2-100, Jan. 16	1	1 0	1	6
Contra Costa	2, apricot	Unsprayed	62	2 8	174	61
		Calcium arsenite 3-100, Jan. 13	8	1 5	12	2
		Calcium arsenite 2-100, Jan. 13	12	1 3	16	3
	3, apricot	Unsprayed	53	6 4	339	49
		Calcium arsenite 2 100, Feb. 9	26	3 3	86	1
	4; apricot	Unsprayed	51	4 5	230	18
		Calcium arsenite 3-100+1 percent oil, Feb. 1	6	2 0	12	2
Sacramento		Calcium arsenite 2 100+1 percent oil, Feb. 1	10	2 2	22	5
	5, plums	Unsprayed	53	3 5	186	26
		Calcium arsenite 2-100, Jan. 24	7	1 7	12	4
		Calcium arsenite 2-100+bordeaux mixture 4-2-100, Jan. 24	19	2 3	44	6
		Calcium arsenite 1-100, Jan. 24	23	2 6	60	13
Tehama	6; apricot [1]	Unsprayed	43	5 7	245	76
		Calcium arsenite 3-100, Jan. 22	1	3.4	3	7
	7, apricot [1]	Unsprayed	31	5 0	155	37
		Calcium arsenite 3-100, Jan. 10	2	1 0	2	8
Stanislaus	8; apricot [1]	Unsprayed	67	5 2	348	22
		Calcium arsenite 2½-100, Jan. 22	12	2 2	26	4
	9, apricot [1]	Unsprayed	65	5 3	344	20
		Calcium arsenite 2½-100, Jan. 18	19	2 3	44	11
Ventura	10, apricot [1]	Unsprayed	25	8 3	208	35
		Calcium arsenite 2 100, Feb. 7	4	1 5	6	4
	11; apricot [1]	Unsprayed	64	3 3	211	20
		Calcium arsenite 2-100, Feb. 8	12	1.4	17	9
Alameda	12, apricot [1]	Unsprayed	55	7 9	435	25
		Calcium arsenite 3¾-100, Jan. 23	16	3.5	56	4

[1] Because of sparse blossom development in these orchards, data on the disease were secured by counting the number of blighted twigs on 5 uniform-sized branches in each tree.

TABLE 5.—*Development of sporodochia in almond and in apricot trees in different orchards sprayed at different dates with various concentrations of calcium arsenite used alone and in combination with other materials, 1940*

ALMOND

County	Orchard No.	Treatment (pounds per 100 gallons) and date sprayed [1]	Plots in different orchards	Twigs with sporodochia (A)	Average number of sporodochia per twig (B)	Index number (A × B)
			Number	*Percent*		
Placer	13	Unsprayed....	1	69	8 8	607
		Calcium arsenite 2-100 + bordeaux mixture 4-2-100, Feb. 1.	1	5	3.3	17
		Calcium arsenite 2-100 + potassium ethyl xanthate 5-100, Jan. 30.	1	9	2 0	18
Sacramento	41	Unsprayed	1	58	4 5	261
		Calcium arsenite 2-100, Jan. 16	1	13	3 1	40
		Calcium arsenite 2-100 + zinc sulfate-lime 4-2-100, Jan. 16	1	15	3 0	45
		Calcium arsenite 2-100 + Hydrolene No. 66,[2] Jan 16	1	14	2 4	34
		Calcium arsenite 1-100 1 percent oil, Jan 16	1	16	2 8	45
	15	Unsprayed	1	78	6 8	530
		Calcium arsenite 2-100, Jan 15	1	9	2 7	24
		Calcium arsenite 1-100 + 1 percent oil, Jan 15	1	16	2 7	43
San Joaquin	16	Unsprayed	1	70	6 3	441
		Calcium arsenite 1 100, Jan 22	1	17	2.9	49
		Calcium arsenite ½-100, Jan 22	1	20	4 9	98
	17	Unsprayed	1	39	4 6	179
		Calcium arsenite 1 100. Jan 20	1	26	4 3	112
	18	Unsprayed	1	25	3 3	83
		Calcium arsenite 1-100, Jan 15	1	7	2 0	14
	19	Unsprayed	1	26	5 2	135
		Calcium arsenite 1 100, Jan. 18	1	14	5 5	77
Stanislaus	20	Unsprayed	1	71	6 6	469
		Calcium arsenite 2½-100, Jan 18	1	15	3 8	57

APRICOT

County	Orchard No.	Treatment (pounds per 100 gallons) and date sprayed [1]	Plots in different orchards	Twigs with sporodochia (A)	Average number of sporodochia per twig (B)	Index number (A × B)
San Joaquin	21-27	Unsprayed	5	66	3 7	244
		Calcium arsenite 4 100	5	6	2 4	14
		Unsprayed	2	74	4 6	340
		Calcium arsenite 3 100	2	14	2 8	39
		Unsprayed	1	92	3 5	322
		Calcium arsenite 3-100 + bordeaux mixture 10-10-100.	1	48	2 1	101
Santa Cruz	28	Unsprayed	1	65	7 2	468
		Calcium arsenite 3 100	1	48	3 8	182
San Benito	29	Unsprayed	3	32	5 7	182
		Calcium arsenite 3-100	3	1	1 3	1
Alameda	30	Unsprayed	2	53	6 8	360
		Calcium arsenite 2½ 100	2	12	3 2	38
		Calcium arsenite 2-100	2	23	5 2	120
Stanislaus	31	Unsprayed	2	51	4 2	214
		Calcium arsenite 2½-100	2	11	2 2	24
Solano	32	Unsprayed	1	67	4 5	302
		Calcium arsenite 2½-100	1	24	2 6	62
		Calcium arsenite 2-100	1	19	3 4	65
Monterey	33	Unsprayed	4	55	4 9	270
		Calcium arsenite 3-100	4	11	2 8	31

[1] The date of application differed among the apricot orchards listed in this table. Except in Solano County, where spraying was done on February 9, applications in the apricot orchards were between January 15 and 22.

[2] Hydrolene No. 66, an organic wetting agent, was added to calcium arsenite, with a little water, in the proportions of 1-5. The mixture was boiled for 30 minutes, dried, and ground to a fine powder. The powder was suspended in water to give arsenite equivalent to 2-100 calcium arsenite.

The inoculum level, as expressed by the sporodochia indices of unsprayed plots (tables 4 and 5), varied greatly in the different orchards. In order, therefore, to evaluate properly the results obtained with various concentrations of calcium arsenite, we must determine whether a given concentration reduced sporodochial development as effectively at the higher levels as at lower ones. When index numbers of individual treatments are assembled and plotted against increasing values of their check index numbers, the results are a series of curves with definite upward trends. That is, in a number of orchards where sporodochia developed in various degrees of abundance, suppression by a given calcium arsenite concentration decreased somewhat with increasing abundance of sporodochia. Under such circumstances, therefore, the suppressive efficacy of the various concentrations will be more accurately expressed by referring treatment index numbers to their check index numbers. A satisfactory method is to divide the treatment index number into the check index number. The primary purpose, however, that of comparing 3–100 and 2–100 calcium arsenite, can be served by plotting one against the other, placing increasing values of check index numbers along the horizontal scale.

The curves in figure 4 are obtained by grouping the control index numbers into a few large classes and averaging the treatment index numbers falling into each class, thus minimizing minor variations. According to these data, 3–100 calcium arsenite is somewhat more effective than 2–100, particularly where sporodochial abundance is greatest. Plots receiving 2½–100 were not numerous enough to be included in the figure, but the results on the whole were about the same as for 2–100.

Calcium arsenite 4–100 in five trials in San Joaquin county apricot orchards (table 5) consistently suppressed sporodochial development to a very high degree, the average reduction being 94 percent. It is noteworthy, however, that 3–100 and 2–100 concentrations, applied in other apricot orchards where the sporodochial level was not greatly different from that in the five orchards sprayed with 4–100, reduced sporodochia 98 and 92 percent respectively. Out of some 30 apricot plots (tables 4 and 5) only one instance approaching failure was noted. This occurred in Santa Cruz County in an orchard (table 5) where sporodochial development was extremely abundant and where, apparently, the spraying was not thorough.

A concentration of 1–100 (without oil) was used in four almond orchards (table 5) and one plum orchard (table 4). Efficiency in the plum orchard was probably impaired by rains occurring before the material dried on the tree. For the almond orchards located in San Joaquin County, 1–100 calcium arsenite gave variable results (table 5). In one case, improper application was suspected because of the manner in which sporodochia occurred in sprayed trees. They abounded on one side of the tree—probably the leeward .during application—and were markedly less abundant on the opposite side. Similar differences were not noted in the check trees. Sporodochial suppression in the other San Joaquin County orchards was decidedly more efficient. Even 1/2–100, applied in one of these orchards, showed definite eradicant effects (orchard 16, table 5).

Keitt and Palmiter (*14*) tested the effect of adding various pro-
portions of copper sulfate and lime to calcium arsenite. They con-
cluded that injury to apple trees was reduced by large amounts of
lime and that the eradicant effect against *Venturia inaequalis* in leaves
was, at the same time, reduced.

In the present studies, bordeaux mixture was added to calcium
arsenite primarily to reduce the arsenical injury, which was particu-
ularly severe on almonds. As arsenite injury to the trees will be
discussed later, the present discussion is confined to the effect of a
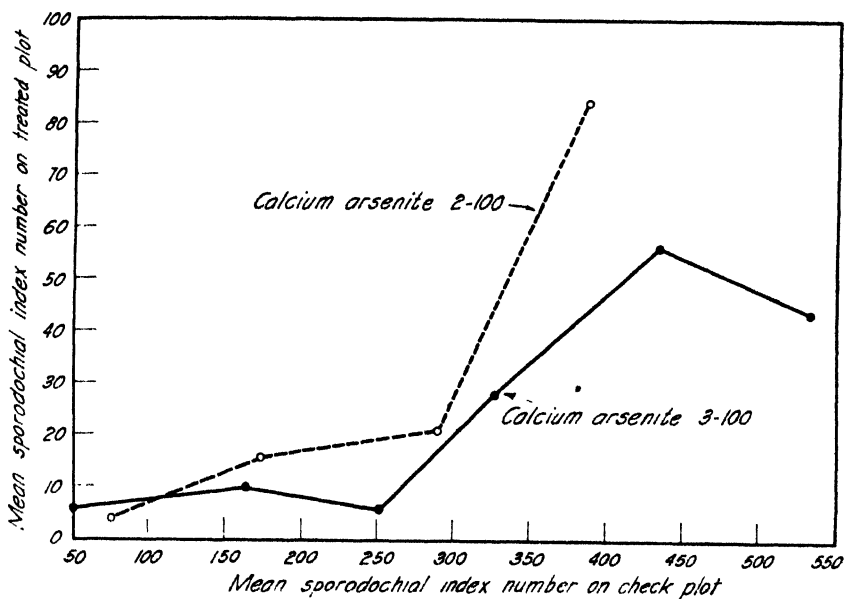
FIGURE 4.—Comparative effectiveness of two concentrations of calcium arsenite
in suppressing sporodochial development at different levels of sporodochial
abundance, 1940. The sporodochial index number was obtained by multiply-
ing the percentage of twigs bearing sporodochia by the average number of these
structures per twig. The higher the index the greater is the abundance of
sporodochia.

weak bordeaux on the eradicant properties of various concentrations
of the arsenite.

Bordeaux was prepared in the spray tank; and the calcium arsenite
was then added. This procedure, of course, is less favorable to the
formation of copper arsenite than that in which the copper sulfate
and calcium arsenite are placed in the spray tank before lime is added.
Though the chemistry of this combination was not studied in detail,
bordeaux was found to reduce the amount of water-soluble arsenic
in the spray mixture.

Turning now to the comparative effectiveness of calcium arsenite
alone and in combination with bordeaux mixture, we see from table
2 that in 1939 bordeaux mixture 8-4-100 sometimes reduced the

efficiency of the arsenite. According to the summarized results in figure 3, the reduced efficiency occurred only when the concentration of calcium arsenite was 2–100 or lower. In many cases, calcium arsenite 2–100 plus bordeaux mixture 8–4–100 appeared only slightly less effective than calcium arsenite 2–100 without bordeaux. If prevention of injury to the tree by this concentration of arsenite were the primary concern, the addition of bordeaux mixture would be desirable. Other aspects of this combination will be discussed later.

In 1940 the addition of 4–2–100 bordeaux mixture to calcium arsenite 2–100 decreased somewhat the suppression of sporodochia in plum trees (table 4, orchard 5). The same combination, however, proved satisfactory in almond trees (table 5). Zinc sulfate and lime at the rate of 4 and 2 pounds, respectively, to 100 gallons of water, decreased only slightly the efficacy of 2–100 calcium arsenite (table 5). According to laboratory tests both bordeaux mixture and zinc-lime reduce the water-soluble arsenic in calcium arsenite. The inadvisability of adding concentrated bordeaux mixture is evidenced in table 5.

Petroleum-oil-emulsion in the 1938 tests (table 1) appeared to increase slightly the efficacy of calcium arsenite in preventing sporodochial development. The large amounts of oil used in these tests were not deemed necessary in later tests. In fact, 4 percent of oil appears to increase the injuriousness of calcium arsenite to apricot trees. Such is the case, for example, in the December 1938 treatments in orchard 11 (table 2). In these particular tests, neither 4 percent nor 2 percent of oil increased the eradicant properties of 4–100 calcium arsenite. According to the summarized data in figure 3, furthermore, 2 percent-oil emulsion affected 4–100 calcium arsenite little if any, and 2–100 only slightly. On the other hand, it apparently increased somewhat the efficiency of 1–100 calcium arsenite.

Petroleum-oil emulsion was added to calcium arsenite in only a few cases in 1940. It was used with 1–100 calcium arsenite in trials on almond trees (table 5), but no parallel treatments without the oil were made in the same orchard. The suppressive power of calcium arsenite 1–100 (without oil) in orchard 16 (table 5) was fully as great as that of the same concentration with oil in orchards 14 and 15. The poorer results obtained with 1–100 in orchard 17 were probably, as noted earlier, the result of improper application.

Potassium ethyl xanthate apparently had no material effect on calcium arsenite as regards either eradication of the fungus or injury to the tree (table 5, orchard 13).

Hydrolene No. 66 was added to calcium arsenite at the rate of 1 pound to 5 pounds of the latter, and the mixture was boiled in a little water for 30 minutes. After filtering and drying, the material was ground to a fine powder, which proved easily wettable and low in water-soluble arsenic. As laboratory tests showed, when a water suspension of this material was aerated for 2 or 3 days, the water-soluble arsenic rose to about the same level as that in untreated calcium arsenite. According to table 5 (orchard 14), this material used in an amount equivalent to 2–100 calcium arsenite was fully as effective as untreated calcium arsenite in suppressing sporodochia, but, as noted later, it proved no less injurious to the trees. Ap-

parently, therefore, it possesses no special qualities to warrant the extra expense of preparing it.

EFFECT OF APPLYING THE SPRAYS IN DECEMBER AND JANUARY

Under California conditions, autumn or early winter is much more favorable for the application of sprays than midwinter or later Since spraying operations are ordinarily not delayed by heavy rains until late December, it would be advantageous to spray in autumn. The likelihood, however, that eradicant sprays would seriously injure trees in leaf prevents application until after leaf fall. Spraying cannot, in consequence, be done before the middle of November, and frequently much later.

In two apricot orchards and one almond orchard various combinations of calcium arsenite were applied a few weeks after leaf fall in December 1938; the same treatments were applied to other trees in mid-January 1939. Since few sporodochia developed on unsprayed trees until after the January applications, the effectiveness of these applications was judged on the basis of their suppression of sporodochial development.

Judging from the results in table 2, applications at both times successfully reduced sporodochial development. When, however, the results are compared in more detail, as in figure 5, certain differences are evident. Whereas December and January applications of calcium arsenite at the rate of 4–100 were equally effective, December applications of 2–100, and particularly 1–100, were less effective than January applications. In three orchards where these trials were conducted, December applications injured the tree much more than January applications. This injury to the host is taken up in a later section of the paper.

EFFECT OF CALCIUM ARSENITE ON THE MYCELIUM OF SCLEROTINIA LAXA IN HOLD-OVER TWIGS

To obtain further information concerning the effects of calcium arsenite on *Sclerotinia laxa*, cultures were made from 25 to 30 hold-over twigs collected from sprayed and unsprayed trees 14 or more days after the sprays were applied. The surface of the twigs was treated for 2 minutes with 1–2,000 mercuric bichloride and then thoroughly washed in sterile, distilled water. The periderm of the twigs was carefully cut away with a sterile scalpel, and sections of the underlying tissue were planted on plates of potato-dextrose agar.

According to data secured during 3 years (table 6), the percentages of unsprayed twigs yielding the fungus were low in a number of cases. In fact, the number of twigs yielding the fungus in culture was lower than the number producing sporodochia. Evidently, therefore, the fungus was not obtained in culture from certain unsprayed twigs in which it was present and viable. Saprophytic fungi, present both on the surface and in the outer tissues of the twigs, appeared frequently to inhibit *Sclerotinia laxa* from developing in culture. Those present in the tissue cannot be killed by surface sterilization, and, as a consequence, sometimes overrun the plates before *S. laxa* can develop. The amount of surface sterilization possible without killing *S. laxa* in twig tissue proved to be limited, 2 to 3 minutes with 1–2,000 mercuric

bichloride being not entirely without effect on it, and yet not always sufficient to kill all surface saprophytes.

The results of isolation studies in Table 6, however, are of value in that they support the data on sporodochial suppression given in the foregoing sections. The percentage of sprayed twigs with viable mycelium was always less than the corresponding value for unsprayed
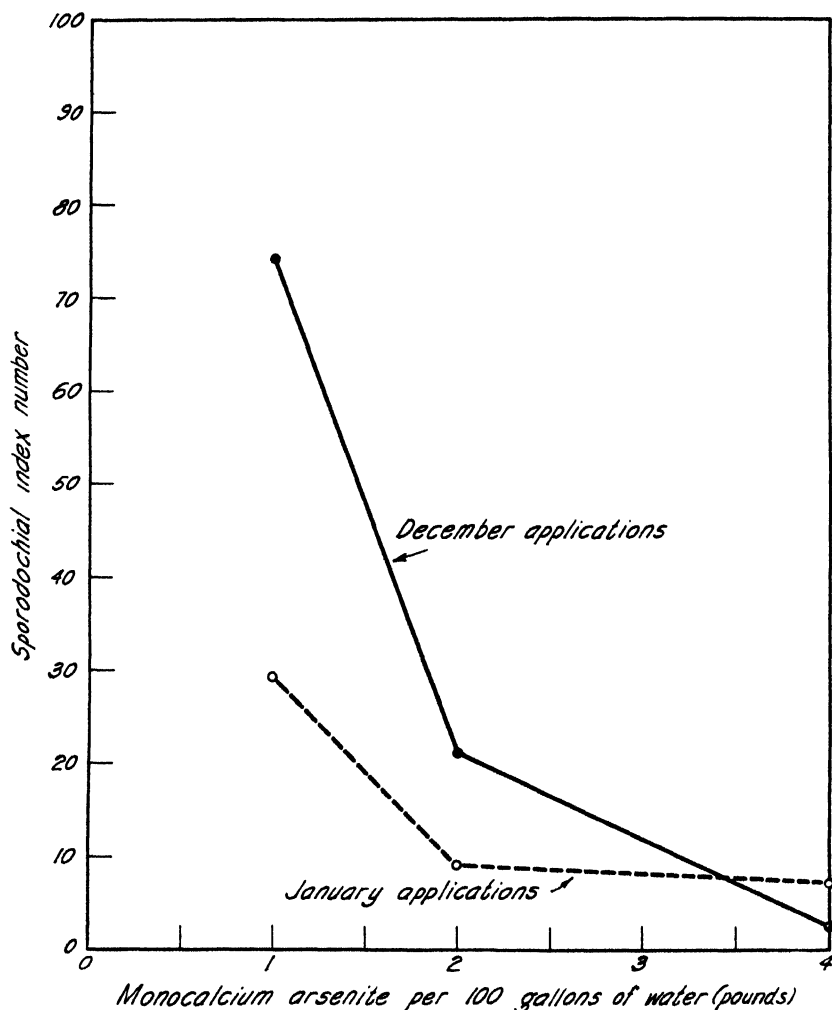
FIGURE 5.—Relation of date of application of calcium arsenite to effectiveness in suppressing sporodochial development in the winter of 1938–39. As the sporodochial index number is the product of the percentage of twigs with sporodochia and the average number of sporodochia per twig, the higher the index number the poorer is the suppression.

twigs. Thus, in three series of isolations from almond twigs in 1938, calcium arsenite 4–100 plus 4 percent oil was found to reduce the percentage of twigs with live fungus 61, 77, and 73 percent. In 1939

the reduction was as follows: Calcium arsenite 4–100 on apricot and almond twigs, 92 and 100 percent, respectively; calcium arsenite 4–100 plus 2 percent oil on apricot, a'mond, and prune twigs, 67, 91, and 90 percent, respectively; and calcium arsenite 4–100 plus bordeaux, 8–4–100, on apricot and almond twigs, 75 and 86 percent, respectively. In 1940 the percentage reductions were, for calcium arsenite 4–100 on apricot twigs, 80, and calcium arsenite 2–100 on almond twigs, 63.

TABLE 6.— *Development of mycelium of Sclerotinia laxa in blighted twigs of almond, apricot, and prune trees sprayed at different dates with two concentrations of calcium arsenite used alone and in combination with other materials, 1938–40*

Year	Kind of trees	Treatment (pounds per 100 gallons) and and date sprayed	Date of isolation	Twigs with live mycelium	Reduction due to spraying
				Percent	Percent
		Unsprayed	Mar. 2	92	
		Calcium arsenite 4–100+4 percent oil Jan 25	do	36	61
1938	Almond	Unsprayed	Mar. 4	73	
		Calcium arsenite 4–100+ percent oil Jan. 25	do	17	77
		Unsprayed	Mar. 8	75	
		Calcium arsenite 4–100+4 percent oil Jan. 25	do	20	73
		Unsprayed	Dec 26	60	
	Apricot	Calcium arsenite 4–100 Dec. 5	do	5	92
		Calcium arsenite 4–100+2 percent oil Dec 5	do	20	67
		Calcium arsenite 4–100+bordeaux mixture 8 4–100 Dec. 5.	do	15	75
1939	Almond	Unsprayed	Dec 27	64	
		Calcium arsenite 4–100 Dec 13	do	0	100
		Calcium arsenite 4–100+2 percent oil Dec. 13	do	6	91
		Calcium arsenite 4–100+bordeaux mixture 8-4 100 Ded. 13	do	9	86
	Prune	Unsprayed	Dec. 30	40	
		Calcium arsenite 4–100+2 percent oil Dec. 12	do	4	90
	Almond	Unsprayed	Feb. 24	51	
1940		Calcium arsenite 2-100 Jan 15	do	19	63
		Calcium arsenite 2- 100+Hydrolene No. 66 Jan. 15	do	17	67
	Apricot	Unsprayed	Mar 1	69	
		Calcium arsenite 4–100 Feb 5	do	14	80

If, for a given treatment, we compare the percentage of twigs with live mycelium with the percentage of twigs producing sporodochia, we find the latter to be the smaller. In 1938, for example, on March 8 the fungus was viable in 20 percent of the twigs sprayed with calcium arsenite 4–100 plus 4 percent of oil, whereas only 2 percent of these twigs bore sporodochia at that time. As the same trend was noted in other years, the inference is that, though the spray did not kill all of the myselium in certain twigs, it did prevent sporodochial development on these twigs. Microscopic examinations of cross sections through unsprayed twigs show the mycelium of *Sclerotinia laxa* to be most abundant in the bark tissues, but present to some extent in the wood and even the pith. Possibly, therefore, the arsenite did not kill all the mycelium in the wood and pith, but did kill it in the bark tissues. As it is the mycelium in bark tissue that produces the sporodochia, killing of this mycelium probably prevents the development of sporodochia.

EFFECT OF ZINC ARSENITE ON SPORODOCHIAL DEVELOPMENT

Zinc arsenite (buffered) was applied at concentrations of 2–100 and 4–100 in seven orchards, and ½–100 and 1-100 in one orchard. As the data in table 7 indicate, its effect on sporodochial development varied considerably. In orchards 11, 12, and 9, applications of 4–100

appeared effective; but elsewhere the same strength gave poor results. Oil apparently increased the effectivene s of the 4–100 concentration in orchard 5, but was not beneficial in orchard 12. On the whole, zinc arsenite possesses much poorer eradicant properties than calcium arsenite. This conclusion grees with Keitt and Palmiter's *(14)* statement that even at 1-percent strength (8–100, approximately) the material did not always satisfactorily suppress perithecial development by *Venturia inaequalis*, whereas calcium arsenite was effective at ½ percent.

TABLE 7.— *Development of sporodochia and of blossom blight in apricot and in almond trees in different orchards sprayed at different dates with various concentrations of zinc arsenite used alone and in combination with other materials, 1938–39*

County	Orchard No. and kind of trees	Treatment (pounds per 100 gallons) and date sprayed	Twigs with sporo- dochia (A)	Average number of sporo- dochia per twig (B)	Index No. (A × B)	Blossom- ing twigs blighted
			Percent			*Percent*
Sacramento	10, almond	Unsprayed	85	6 8	578	33
		Zinc arsenite 4-100, Jan. 17	34	3.9	133	15
		Zinc arsenite 4-100+bordeaux mixture 8-4-100, Jan 17	67	4 1	275	26
		Zinc arsenite 2-100, Jan. 17	53	3 8	201	23
		Zinc arsenite 2-100+2 percent oil, Jan. 17	56	4 7	263	31
Contra Costa	11, apricot	Unsprayed	70	8 4	588	27
		Zinc arsenite 4-100, Dec. 5	20	2.2	44	1
		Zinc arsenite 4-100, Jan 18	23	2 6	60	2
		Zinc arsenite 4-100+bordeaux mixture 8-4-100, Jan 18	55	3 1	171	3
		Zinc arsenite 2-100, Jan. 18	48	2 2	106	5
		Zinc arsenite 2-100+bordeaux mixture 8-4-100, Jan 18	65	5 6	364	4
Stanislaus	7, almond	Unsprayed	43	4 6	198	56
		Zinc arsenite 4-100+2 percent oil, Jan. 19	37	1 7	63	28
		Zinc arsenite 4-100+bordeaux mixture 8-4-100, Jan. 19	33	2 0	66	31
		Zinc arsenite 2-100, Jan. 19	45	3 0	135	42
		Zinc arsenite 2-100+bordeaux mixture 8-4-100, Jan. 19	43	2 4	103	43
	5, apricot	Unsprayed	70	9 4	658	46
		Zinc arsenite 4-100, Jan 20	64	5. 8	371	26
		Zinc arsenite 4-100+2 percent oil, Jan. 20	18	1 6	29	12
		Zinc arsenite 4-100+bordeaux mixture 8-4-100, Jan 20	75	6 0	450	
	6; apricot	Unsprayed	48	2. 8	134	55
		Zinc arsenite 4-100, Jan 20	38	3 4	129	18
		Zinc arsenite 4-100+2 percent oil, Jan 20	17	2 3	39	15
	12, apricot	Unsprayed	45	5 8	261	72
		Zinc arsenite 4-100, Jan. 19	40	1 7	68	66
		Zinc arsenite 4-100+2 percent oil, Jan. 19	21	3 7	78	71
San Joaquin	9, apricot	Unsprayed	49	3 9	191	76
		Zinc arsenite 4-100, Jan 19	8	2.0	16	61
	8, almond	Unsprayed	72	5. 5	396	
		Zinc arsenite 1-100, Jan. 19	52	3 7	192	
		Zinc arsenite ½-100, Jan. 19	76	3. 2	243	

Of the three arsenites used—sodium, calcium, and zinc—that of zinc is the least soluble, a fact that may partly account for its low toxicity to the fungus in the present work. Another factor might be a low sticking quality when applied to trees; but such a condition is doubtful inasmuch as this material, applied in winter, caused injury to almond trees as late as June.

EFFECT OF SODIUM ARSENITE ON SPORODOCHIAL DEVELOPMENT

Information at hand showed sodium arsenite in concentrations greater than 1 pound of the salt to 100 gallons of water to be highly toxic even to apricot trees, which are more tolerant of calcium arsenite than almonds. For test purposes, however, the material was used in concentrations of 1, 2, and 3 pounds per 100 gallons of water. In one orchard 4–100 was used.

In the 1938 tests (table 1) 1.2–100 sodium arsenite was ineffective in preventing sporodochial development, but in the 1938–39 tests (table 8) it was more effective except in the earliest (December 29) application. Judging from comparative tests in orchards 3 and 4, 1–100 is much less effective than 2-100. When, however, petroleum-oil emulsion in large amounts was added to the 1–100 concentration in one of these orchards, much better suppression was effected. When this material was stronger than 1-100, injury to the tree was serious.

TABLE 8.— *Development of sporodochia and of blossom blight in apricot and in almond trees in different orchards sprayed at different dates with various concentrations of sodium arsenite used alone and in combination with other materials, 1938–39*

County	Orchard No. and kind of trees	Treatment (pounds per 100 gallons) and date sprayed	Twigs with sporodochia (A)	Average number of sporodochia per twig (B)	Index No. (A×B)	Blossoming twigs blighted
			Percent			Percent
Tehama	1 apricot	Unsprayed	49	4.9	240	39
		Sodium arsenite 1-100, Dec. 29	32	3.8	122	31
Alameda	2 apricot	Unsprayed	72	7.5	540	13
		Sodium arsenite 1-100, Feb. 8	23	1.9	44	1
	3 apricot	Unsprayed	82	7.2	590	13
		Sodium arsenite 3-100, Feb. 8	13	3.1	40	1
		Sodium arsenite 2-100, Feb. 8	19	2.4	46	2
		Sodium arsenite 1-100, Feb. 8	27	3.3	89	2
Monterey	4 apricot	Unsprayed	79	10.1	798	----
		Sodium arsenite 3-100, Jan. 30	5	1.9	10	---
		Sodium arsenite 2-100, Jan. 30	8	2.3	18	---
		Sodium arsenite 1-100, Jan. 30	32	4.1	131	---
		Sodium arsenite 1-100+5 percent oil, Jan. 30	16	3.0	48	
Stanislaus	5 apricot	Unsprayed	70	9.4	658	46
		Sodium arsenite 1-100, Jan. 20	8	1.5	12	11
	6 apricot	Unsprayed	48	2.8	134	55
		Sodium arsenite 1-100, Jan. 20	9	2.3	21	8
	7 almond	Unsprayed	43	4.6	198	56
		Sodium arsenite 1-100, Jan. 19	20	2.2	44	30
		Sodium arsenite ½-100, Jan. 19	24	2.8	67	22
San Joaquin	8 almond	Unsprayed	72	5.5	396	----
		Sodium arsenite 1-100, Jan. 18	18	2.5	45	---
		Sodium arsenite ½-100, Jan. 18	28	3.1	87	---
	9 apricot	Unsprayed	49	3.9	191	76
		Sodium arsenite 4-100, Jan. 19	1	1.5	2	36

EFFECT OF ARSENITES ON CONIDIA BORNE ON SPORODOCHIA PRESENT WHEN SPRAYS ARE APPLIED

Since they occupy a relatively exposed position on the twigs, the sporodochia can be covered by sprays which would destroy either the entire sporodochium or the conidia borne thereon. Destroying the conidia without rendering the sporodochia incapable of producing new spores, or destroying the sporodochia without preventing the development of others, will, of course, cause only a temporary reduction in the abundance of inoculum. In the period of maximum development

new sporodochia may be produced within a few days. If, therefore, a spray, which destroyed these structures but did not prevent their further development, was applied, for example, 2 weeks before the trees bloomed, a new lot of spores could be produced in time to cause serious blossom infection.

Having found that calcium arsenite will prevent sporodochial development, the next step was to determine its effects on the conidia on sporodochia that were present when the spray was applied.

As noted earlier, sporodochia begin to appear on apricot and almond twigs in early January, or in some years in late December (fig. 2), and are abundant by late January or early February. As all sprays listed in the foregoing tables were applied before the sporodochia developed in large numbers, others were applied in early February to determine their effects on the conidia. At the blossoming period, or shortly after, twigs collected from sprayed and unsprayed trees were brought to the laboratory. The conidia from 25 or 30 sporodochia were washed off into separate lots of double-distilled, sterile water. By adding more water the number of spores per unit volume of each lot of suspension was adjusted to about the same value. Three drops of each suspension were placed in separate places on freshly prepared potato-dextrose agar in Petri dishes. For most reliable results—as shown by preliminary trials—observations on germination must be made after about 15 hours' incubation at 25° C. A shorter period does not allow time for all spores to germinate, and a longer period permits the germ tubes to become so long as to interfere with observations.

In addition to the germination tests, sporodochia and the conidia were examined microscopically for evidence of injury.

Almond trees sprayed on February 17, 1938, with calcium arsenite 3–100 were examined on February 25. As shown in table 9, 74 percent of the conidia from unsprayed trees germinated, whereas only 4 percent of the conidia from sprayed trees germinated.

Zinc arsenite 3–100 and zinc arsenite 3–100 plus 2 percent oil, applied to almond trees on February 23, 1938, had greatly reduced germinability of the conidia by March 1 (table 9).

The 1939 plan was to spray apricot trees in orchard 11 and almond trees in orchard 14 with all the materials used in January (table 2), but wet soil prevented this to a large extent. Certain treatments, however, were given in these orchards and in almond orchard 16 on February 9 and 15.

Material for germination tests was collected from orchard 14 on February 13, 15, and March 20, from orchard 16 on February 20 and March 20, and from orchard 11 on February 21 and March 17. As the results obtained at the different dates agreed in all details, only those of March 17 and 20 are given in table 10.

The most noticeable feature of the data in table 10 is the failure of calcium arsenite to effectively kill the conidia. A concentration of 2–100 in orchard 14 reduced germinability only 39 percent. Two percent of oil added to calcium arsenite 1–100 seemed to increase the killing power of the latter, in that conidial germinability was reduced 75 percent. The same treatment in orchard 11 reduced germinability 69 percent.

TABLE 9.—*Effect of calcium and zinc arsenite sprays on conidia borne on the sporo-dochia present when sprays were applied and on subsequent blossom blight in almonds, 1938*

Orchard No.	Treatment (pounds per 100 gallons) and date sprayed	Trees observed	Conidia germinating [1]	Blossoming twigs blighted
		Number	*Percent*	*Percent*
	Unsprayed..	9	74	62
	Calcium arsenite 3-100, Feb. 17	8	4	19
	Unsprayed	5	84	24
	Zinc arsenite 3-100, Feb. 23	5	7	8
	Zinc arsenite 3-100+2 percent oil, Feb. 23	5	8	4

[1] Tests of conidial germinability were made on Feb. 25 in orchard 1 and on Mar. 1 in orchard 2.

TABLE 10.—*Effect of calcium and zinc arsenite sprays on conidia borne on the sporodochia present in almond and apricot trees when sprays were applied, and on subsequent blossom blight, 1939*

County	Orchard No. and kind of trees	Treatment (pounds per 100 gallons) and date sprayed	Conidia germinating [1]	Blossoming twigs blighted
			Percent	*Percent*
		Unsprayed	51	40
		Calcium arsenite 2-100, Feb. 9	31	24
		Calcium arsenite 2-100+bordeaux mixture 8-4-100, Feb. 9.	24	21
Sacramento	14, almond	Calcium arsenite 1-100, Feb. 9	20	30
		Calcium arsenite 1-100+2 percent oil, Feb. 9	13	15
		Zinc arsenite 2-100, Feb 9	39	35
		Calcium arsenate 4-100, Feb 9	34	34
Sacramento	16, almond	Unsprayed	66	53
		Calcium arsenite 1-½-100+ Elgetol [2] 1 percent Feb 15.	47	42
Contra Costa	11; apricot	Unsprayed	71	36
		Calcium arsenite 1 100+2 percent oil, Feb 15	22	8

[1] Tests of conidial germinability were made on Mar. 20 in orchards 14 and 16, and on Mar. 17 in orchard 11.
[2] Elgetol, a proprietary spray material containing 24 percent sodium dinitro-o-cresylate.

The low toxicity of these treatments is in marked contrast to the high toxicity of those given in orchard 14 (table 2) on January 17, at which time a few sporodochia were present. Germination tests on February 13 showed that the January 17 application of calcium arsenite 2-100 had reduced conidial germinability 98 percent. In fact, this treatment injured all sporodochia present at the time, many being so badly injured that the discolored, collapsed spores could be identified only by carefully separating them from the sporodochium with teasing needles. Furthermore, zinc arsenite 2-100, which in the February application (table 10) reduced conidial germinability only 24 percent, in a January 17 application (table 7) reduced germinability 65 percent.

A lack of moisture to render the arsenic soluble is believed to have been at least partly responsible for the low toxicity of the February 9 applications. Whereas rain occurred at intervals after the January 17 applications, the weather was comparatively dry throughout the last 3 weeks of February and the first week of March. Sections through sporodochia from twigs sprayed February 9 with calcium arsenite 2-100 (table 10) showed that the outermost conidia had been injured, but those in the center were normal and capable of germinating.

Keitt and Palmiter (*14*) recognized the importance of moisture to the toxic action of the copper-lime arsenites.   As noted in a previous section of this paper, the addition of copper sulfate and lime (bordeaux mixture) to calcium arsenite reduced the water-soluble arsenic in the spray.   Hence, even more prolonged wetting of copper-lime arsenite than of calcium arsenite would seem necessary to produce soluble arsenic in amounts sufficient to kill spores.   Though no evidence on this point is at hand, the fact that zinc arsenite, the most insoluble of the arsenites used here, was less efficacious than calcium arsenite, both in preventing sporodochial development and in killing conidia, seems to support the view that rain, or moisture in other forms, is highly important to the toxicity of these materials.

In the following instance solubility seems to have been an important factor in toxicity.   On February 21, 1939, applications of sodium arsenite at the rate of 1–100 and zinc arsenite at the rate of 4–100 were made to apricot trees.   During the rest of February no rain fell, and there were only 2 days of rain before March 14, at which time germination tests were made.   Under these conditions zinc arsenite reduced germinability of conidia only 37 percent, whereas sodium arsenite reduced it 91 percent.   That is, the highly soluble sodium arsenite was the more toxic, even though at the concentrations used the total arsenic was greater in the case of the less soluble zinc arsenite.

Sodium arsenite, it will be recalled, was somewhat inconsistent in preventing sporodochial development.   Possibly its high solubility accounts for this inconsistency.   Heavy rains occurring shortly after application may wash it from the tree before it enters the bark and kills the mycelium of *Sclerotinia laxa*.   At 3–100 and 2–100 concentrations it successfully prevented the development of sporodochia in two orchards (Nos. 3 and 4, table 8), and at a 1–100 concentration it effectively prevented their development in a number of cases where applications were made in late January or early February.   The least effective application was that made December 29 in orchard 1 (table 8).   Possible, therefore, weather conditions may account for the inconsistencies.

EFFECT OF A REDUCTION IN INOCULUM ON SUBSEQUENT BLOSSOM INFECTION

Because of uncertainty regarding injury by the materials, many of the tests in 1938 and 1939 and some of those in 1940 were conducted on a small number of trees.   As will be noted later, the blossom infection in sprayed trees was sometimes increased by spores that drifted into them from nearby unsprayed trees.   Hence, small plots were not so suitable for testing the effects of the sprays on the disease as were larger plots.   This was true particularly if unsprayed trees were present to windward of small sprayed plots.   In the following discussion cases will be noted where poor control was attributable to spores originating outside the treated trees.

In a strict sense, the incidence of the disease is measured by the percentage of blossoms actually invaded.   The data on disease severity obtained in these studies were not based on the percentage of blossoms attacked for the following reasons.   Upon entering a blossom the fungus may quickly grow into the spur or terminal|on which the blossom is borne.   Shortly after the invaded blossom shows the first signs of the disease, other blossoms on the twig, such as those in the same

cluster, and those distal to the infected blossom, die because the twig is girdled. Unless observed just before they die the blossoms killed by girdling are difficult to distinguish from those invaded by the fungus. When tests are extensive and are located in widely separated districts where blossom infection occurs at different times, each orchard cannot be examined at the proper time. In the present studies, therefore, observations were delayed until blighting of twigs was accomplished, thus allowing the symptoms to reach the same stage in all orchards regardless of when infection occurred.

Each blossom-bearing spur and terminal was considered a unit and the percentage of these units blighted was determined by counting 200 at random in each tree. An abnormal blossom development in 1940 necessitated a second method for taking records in certain districts. Blighted spurs and terminals were counted on five selected branches in each tree.

These methods of recording disease occurrence, of course, do not yield strictly quantitative data. The sporodochial index numbers, it will be recalled, measure the frequency of sporodochia (and consequently conidia) on the hold-over twigs, but do not indicate the total number in a tree. In view of these facts we must not expect strictly proportional relations between the two sets of data. On the whole, the disease index number will be high when the sporodochial index number is high, and low when it is low, but to obtain similar control in two given orchards where the number of hold-over twigs differs greatly, the efficiency of sporodochial supression must be higher in the orchard with the greater number of hold-over twigs. Hence, we may expect to find cases where the same degree of sporodochial supression results in different control.

The relations between primary inoculum level and disease level may possibly be disturbed by still another factor. As infected blossoms produce conidia soon after they wilt, these conidia might be responsible for further blossom infection. If such is the case, a few infected early blossoms may produce sufficient conidia to cause a severe outbreak of the disease in blossoms which open later, even though the eradicant spray had efficiently suppressed the primary inoculum. Though secondary infection of this sort is probably of minor importance in a warm spring, it might be important in years when low temperatures or other factors prolong the blossoming period. Except under some such conditions the period of blossom susceptibility, extending from emergence of the blossoms to petal fall, is so short that the earliest infected blossoms scarcely have time to produce conidia before the petals are off all blossoms.

BLOSSOM BLIGHT IN TREES SPRAYED BEFORE MANY SPORODOCHIA HAD DEVELOPED

In 1938 significant reduction in severity of blossom infection (table 1) accompanied the application of calcium arsenite before sporodochia appeared. Less important reduction followed applications of zinc and sodium arsenite. The relation between sporodochial abundance and disease severity is seen when the data in table 1 are represented as percentage sporodochial prevention and percentage disease reduction. Thus, calcium arsenite 4–100 reduced sporodochia 95 percent and the disease 77 percent; calcium arsenite 4–100 plus 4 percent of oil, 99

and 79 percent, respectively; zinc arsenite 4–100, 88 and 52 percent; zinc arsenite 4–100 plus 4 percent of oil, 80 and 57 percent; and sodium arsenite 1.2–100, 33 and 16 percent.

The 1939 trials were conducted under a variety of climatic conditions and in orchards where the past history of the disease varied considerably, and in which, as we saw earlier, production of inoculum by the fungus differed greatly. Hence, we may expect variabilities in the results. According to data in tables 2, 3, 7, and 8, it may be concluded that blossom blight decreased markedly with high degrees of sporodochial suppression, although not invariably. In other cases, furthermore, an equally low incidence of the disease occurred in plots where the degree of sporodochial suppression differed greatly. The cause of these exceptional cases must be sought among factors such as the abundance of hold-over twigs, the presence of wind-borne conidia, and others.

Both calcium and zinc arsenites, for example, gave satisfactory disease control in orchard 11 (tables 2 and 7), though sporodochial suppression by the latter was only a fraction of that by the former. Granting that our knowledge of the factors responsible for these differences in results is not complete, nevertheless several of the more important are known. Where the index numbers show a low degree of sporodochial suppression, either because the material was ineffective or because it had not been applied properly, we should expect little reduction in the disease. Aside from the exceptional control of blossom infection in orchard 11 (table 2), the data conform to this view. Accordingly, it must be concluded that certain differences existed between orchard 11 and other orchards. As noted earlier, the total number of hold-over twigs per tree contributes to the severity of blossom infection: the greater the total number of spores, the greater the chances for infection. Differences in the total hold-over sources arise from differences in severity of the disease the previous year, or from differences in the size of trees, or both. The trees in orchard 11, for example, were smaller than those in many of the other orchards; and, though the incidence of disease had been high in past years, the total number of hold-over twigs per tree was low as compared with that in other orchards. Orchard 11A, located 1 mile from orchard 11, represented a different set of circumstances; the disease was severe in previous years, the trees were large, and, in consequence, the total number of hold-over twigs was greater in these trees than in those of orchard 11. Although in untreated plots of orchard 11 the level of sporodochial development exceeded that in untreated plots of 11A, the incidence of disease was higher in the latter orchard. Parallel treatments of calcium arsenite 4–100 reduced sporodochial abundance 99.5 percent in orchard 11 and 99 percent in orchard 11A; yet the disease was less effectively controlled in the latter than in the former. In other words, the disease was more difficult to control in orchard 11A because it was necessary to obtain a higher relative degree of sporodochial suppression in order to overcome the difference in total sporodochial numbers.

The results secured in orchard 11A, therefore, represent more rigid tests of the efficiency of calcium arsenite. This conclusion does not, of course, invalidate the results secured in orchard 11, for here the significance of disease reduction was attested by the abundance of

blossom blight developing in three unsprayed plots located in different parts of the orchard.

Unsatisfactory control of blossom infection followed application of calcium arsenite in orchards 12 and 9 (table 3) even though suppression of the fungus was highly effective. The plots in these orchards as well as in orchard 5 (table 3) were small and, in consequence, were near untreated trees bearing large numbers of sporodochia. In orchard 9, for example, the plot receiving calcium arsenite 4–100 on November 30 consisted of six trees, two adjacent to the untreated block, and the rest not more than two or three rows from other untreated trees. In orchard 12, treated blocks two rows wide and four trees long were so situated that untreated trees bordered them both on the north and on the south. The treated trees in both orchards were therefore exposed to wind-borne spores from other trees. Evidence that spores from sources outside the treated trees caused infection was present in orchard 9; the two treated trees adjacent to the control trees were more severely infected than the other trees receiving the same treatment.

Additional evidence that spores drift from one tree to another was secured in Contra Costa and Tehama Counties. In two orchards untreated trees were located south of plots receiving calcium arsenite. In both cases the treated trees adjacent to the untreated plot developed blossom blight more abundantly than trees three or four rows away. The effect of drifted spores was also noticeable in the second treated row. During periods of rain at blossoming time, southwest winds with velocities of 14 to 16 miles per hour occurred in these districts. In small plots or in plots located leeward of untreated trees bearing sporodochia it seems not unlikely, therefore, that wind-borne conidia or conidia in wind-borne rain may vitiate the disease control otherwise obtainable with eradicant sprays.

Data in table 2 reveal that the addition of bordeaux 8–4–100 and oil emulsion (2 percent) had no marked effect on disease control by calcium arsenite 4–100 in orchard 11A. Bordeaux, however, somewhat reduced the efficacy of calcium arsenite 4–100 in orchard 14 (table 2) and markedly reduced the efficacy of December applications of 2–100 and 1–100 concentrations.

Calcium arsenite 2–100, alone and with oil, gave excellent control in several instances. Aside from orchard 11 (table 2), where control was good and was easily accomplished, this concentration reduced the disease as follows: In orchard 14 (table 2) December applications, 2–100, 88 percent, and 2–100 plus 2 percent of oil, 79 percent; January applications, 2–100, 82 percent, and 2–100 plus 2 percent of oil, 88 percent. In orchard 5 (table 3), 2–100 plus 2 percent of oil reduced the disease 65 percent.

A December application of calcium arsenite 1–100 in orchard 14 (table 2) reduced the disease 73 percent. December and January applications of calcium arsenite 1–100 plus 2 percent of oil were accompanied by reductions of 70 and 67 percent, respectively. Evidently, therefore, rather dilute suspensions of this material gave significant control.

Except in orchard 11, zinc arsenite was comparatively ineffective against blossom blight. The results in table 7 indicate some control at a 4–100 concentration, but no case equaled the best obtained with calcium arsenite.

Except for orchard 9 (table 8), where the amount of disease in treated plots was apparently increased by spores drifting in from untreated trees, the control with sodium arsenite paralleled, on the whole, the eradicant efficiency. In orchard 1, sodium arsenite 1–100 effected only a moderate reduction in the number of sporodochia, and practically no reduction in blossom infection. In orchards 2, 3, 5, and 6, on the other hand, both sporodochial numbers and incidence of the disease were reduced greatly. In orchard 7, control was noticeable but not satisfactory. The inconsistent results with sodium arsenite may be explained, in part, by its high solubility. It apparently has considerable immediate effect on sporodochia present when it is applied, but seems to lose this effect during heavy rains. The composition of sodium arsenite solutions used in these tests probably varied, and this variation may have caused some of the inconsistencies.

As the disease was absent in certain districts in 1940, no data were obtained. Results, however, were obtained in 12 orchards. blossom infection being very abundant in 5 and moderately abundant in 7. Because of abnormal blossom development in some districts two methods were employed in collecting the results. In orchards where the trees blossomed normally, the percentage of blossoming shoots blighted was determined in the same manner as in earlier years (table 4, orchards 1 to 5). In orchards where blossom buds developed abnormally, the number of blighted blossoming twigs was counted on selected branches (table 4, orchards 6 to 12).

Except in orchard 9 (table 4) and in the case of calcium arsenite, 1–100, in orchard 5 (table 4), satisfactory reduction in blossom infection resulted from suppressing the sporodochia. The occurrence of abundant blossom infection in orchard 9 despite rather effective prevention of sporodochial development is believed to have resulted from wind-borne conidia from nearby unsprayed trees. This plot was small and was located leeward of unsprayed trees. In case of the calcium arsenite 1–00, sporodochial suppression was not highly effective, possibly because rains occurred before the spray had thoroughly dried.

In Contra Costa and Tehama Counties where the disease was severe, blossom blight was reduced 85 percent in orchard 1, 97 and 95 percent in orchard 2, 98 percent in orchard 3 (table 4), 91 percent in orchard 6, and 78 percent in orchard 7 (table 4). Through the data are not numerous enough to allow comparisons of various concentrations, they suggest that both 3–100 and 2–100 were highly effective under the 1940 conditions.

Opportunity was offered in 1940 for comparing the control given by calcium arsenite alone, calcium arsenite followed by the standard applications of bordeaux in the preblossom stage, and bordeaux in the preblossom stage. The spring of 1940 proved unfavorable for spraying, in that rains occurred frequently during the preblossom stage. A relatively short dry period just as the blossoms emerged from the bud was sufficient to allow certain orchardists to apply bordeaux mixture. The rains responsible for the major part of blossom infection occurred within a few days after application of the spray. In most of these cases bordeaux effectively controlled the disease.

According to data in figure 6, the eradicant sprays, calcium arsenite 2–100, 2½–100, and 3–100, compared favorably with bordeaux 10–10–100, 12–12–100, and 16–16–100 with respect to reduction of

blossom infection.   In three out of four cases, a calcium arsenite spray followed by a bordeaux spray effected moderately better control than when calcium arsenite alone was applied; and in one of the four cases, control was significantly better.   Neither bordeaux alone, calcium arsenite alone, nor calcium arsenite followed by bordeaux gave satisfactory control in one of the six orchards.   In this orchard the calcium arsenite plots were small and, in consequence, exposed to wind-borne conidia from unsprayed trees.
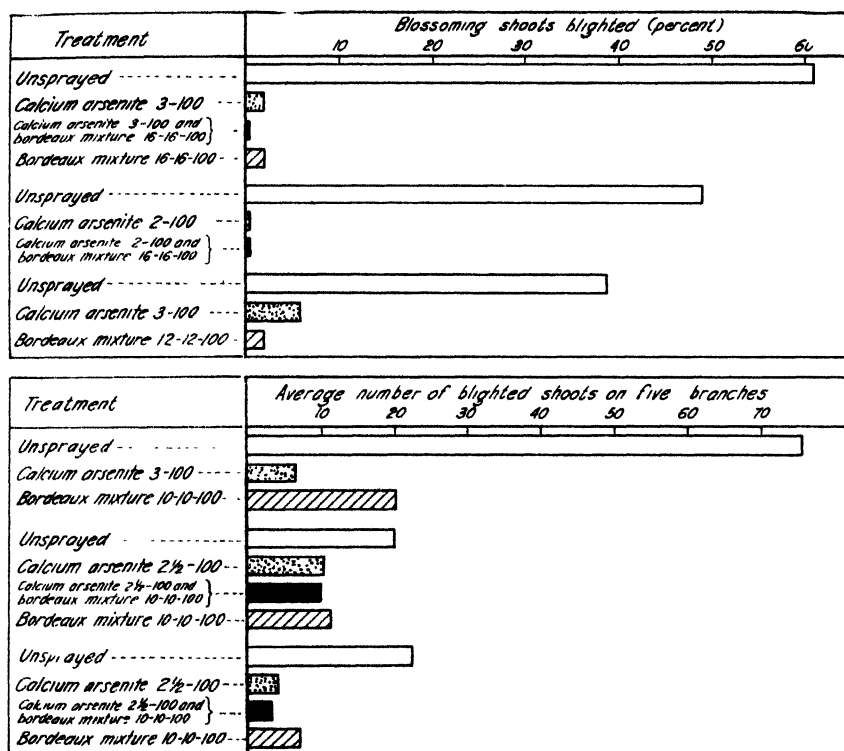
Figure 6.—Control of apricot blossom blight by winter applications of calcium arsenite and by preblossom applications of bordeaux mixture, 1940.   Quantities given in pounds per 100 gallons.

BLOSSOM BLIGHT IN TREES SPRAYED AFTER SPORODOCHIA HAD DEVELOPED IN LARGE NUMBERS

Tables 9 and 10 contain results of the few treatments given when sporodochia were present in the trees in large numbers.   In 1938 the material reductions in conidial germinability attending applications of both calcium arsenite 3–100 and zinc arsenite 3–100 were accompanied by a fairly satisfactory reduction in blossom infection.   In 1939, on the other hand (table 10), inasmuch as the sprays proved ineffective in killing conidia, the disease was not reduced greatly.   An exception was the 78 percent reduction of the disease attending application of calcium arsenite 1–100 plus 2 percent of oil in orchard 11. This orchard, it will be recalled, proved exceptional in cases of all other applications.

INJURIOUS EFFECTS OF THE ARSENITES

Injury to the tree was soon found to limit somewhat the usefulness of arsenites as eradicant sprays. Sodium arsenite, probably because of its high solubility, was more injurious than calcium or zinc arsenite. For this reason, concentrations above 1 pound of the salt per 100 gallons of water were used in only a few instances. Apricots appeared able to tolerate 1–100 sodium arsenite, provided the trees were dormant when sprayed. Almonds proved so highly sensitive to all the arsenites that no concentration effective against the fungus could be used with safety. The varieties of plums and prunes tested appear tolerant towards effective concentrations of calcium arsenite.

Injury was manifested in several ways. Any comparatively fresh wound permits the absorption of soluble arsenic with resultant killing of tissue around the point of entry. Pruning wounds, therefore, were common points through which arsenic gained access to bark tissue. Upon entering the cut ends of branches the arsenic would sometimes kill the bark tissue for a considerable distance from the end. Lateral twigs arising from these killed areas were girdled and killed. When pruning operations were delayed until after application of the spray, much of this injury was avoided, although in certain instances soluble arsenic entered wounds made after the trees were sprayed. Such occurrences, however, did not ordinarily cause material damage.

Arsenic entered directly through the unbroken bark to some extent in almonds, but usually not in apricots. Judging from the effect of arsenites on mycelium in hold-over twigs, one would expect the bark of dead twigs to be highly permeable to soluble arsenic. Some minor injury occurred in almonds, where the arsenic, upon entering dead twigs, was carried downward into the live portions. Such injury was not observed in apricots or plums.

A problem with almond trees was the killing of young shoot growth in early summer by arsenites applied the previous winter. After the first period of high temperatures in late May or early June, clusters of leaves on terminals and spurs suddenly wilted, the cause being the death of tissue at the bases of currently developing twigs. Since the sprays were applied before these twigs developed, injury probably arose from soluble arsenic washed over these tissues by rain. In all cases, light showers preceded the injury by a week or more. Neither apricots nor plums developed this type of injury.

Because arsenites are extremely toxic to plant tissue unprotected by a thick periderm, the eradicant sprays must be applied only while the trees are dormant; that is, between leaf-fall in the autumn and the swelling of buds in the spring. Since autumn weather conditions are favorable for spraying, special advantages would arise from treatment at this time. With this fact in mind, sprays of various concentrations and combinations of calcium arsenite were applied in December 1938 and January 1939. The December sprays were delayed until 2 to 3 weeks after leaf-fall. The relation of the time of application to sporodochial suppression was discussed earlier. In all three orchards—two of apricot and one of almond—December applications produced far more injury than January applications. For example, 4-100 calcium arsenite applied in December killed twigs of ½ to ¾ inch diameter, whereas the same concentration applied in January killed only a few of the smallest twigs. Bud injury, with

consequent reduction of crop, was especially noticeable on trees sprayed in December, but absent from those sprayed in January. The difference was found to be connected with the leaf-scars. For a time after leaf-fall the vascular elements of leaf-scars are apparently accessible to soluble arsenic, which enters and kills the tissues at the base of buds arising near the scars. After December spraying, enough arsenic was absorved through leaf-scars to kill large twigs as well as individual buds on these twigs. By January, however, the leaf-scars were apparently no longer permeable to the arsenic, and bud and twig injury was not produced even by 4-100 calcium arsenite, except where the arsenite entered pruning wounds.

The following observations pertain to the effects of supplementary materials on injuriousness of December applications of calcium arsenite. Two-percent oil emulsion increased injury slightly, whereas 4 percent increased it materially (table 2). Bordeaux mixture 8-4-100 reduced injury of 4-100 calcium arsenite, but not to the point where this combination was safe. Calcium arsenite 2-100 plus bordeaux 8-4-100 produced little injury as compared with calcium arsenite 2-100. Except calcium arsenite 1-100 and its combinations, however, all December applications were far more injurious than January applications.

Zinc arsenite treatments paralleling these calcium arsenite treatments proved much less injurious, the only noticeable damage occurring on almond trees sprayed in December. Since, however, this material possesses low eradicatory properties, there is no reason for preferring it to calcium arsenite.

In the extensive trials of 1940, calcium arsenite caused material injury in only 3 out of about 30 apricot orchards. In one of these 3 cases 4-100 calcium arsenite was applied to trees injured by sodium arsenite the previous season. Four other orchards in the same district sprayed with this concentration developed no injury. In another orchard petroleum-oil emulsion was added to 3-100 calcium arsenite. Branch injury was rather severe. The third case of injury occurred in a Contra Costa orchard sprayed on February 9 with 2-100 calcium arsenite (table 4, orchard 3). At this date the blossom buds were swelling, and arsenic entered and damaged them somewhat. The spray was applied with a large rig operating at about 500 pounds' pressure, which is believed to have driven the spray into the buds, since no injury followed the spraying at 250 pounds' pressure on February 9 in Solano County (table 5).

The primary purpose of the 1940 tests in almond orchards was to determine the injurious effects of calcium arsenite 2 100 and 1-100 alone and combined with other materials (table 5). The following information was obtained: (1) Conditions in 1940 were much more conducive to injury than in 1939. The amount of rainfall following the application was much greater in 1940 than in 1939. (2) Though both zinc-lime 4-2-100 and bordeaux mixture 4-2-100 reduced the injury caused by 2-100 calcium arsenite in the winter, neither noticeably reduced the injury caused in early summer. (3) Potassium ethyl xanthate proved ineffective in reducing injury. (4) Although Hydrolene No. 66, at the rate of 1 pound to 5 pounds of calcium arsenite, temporarily lowered water-soluble arsenic, it did not reduce injury. (5) Even at concentrations of 1-100 the calcium arsenite did consid-

erable damage that appeared in the summer as a withering of leaf clusters.

To determine the effect of bordeaux applied in the preblossom stage on the injury which develops in summer, two blocks of almonds previously sprayed with calcium arsenite 2–100 (table 5, orchards 14 and 15) were sprayed with a 12–12–100 concentration of the material. Injury was reduced but not avoided. Such prevention of injury as did occur was probably the result of the fact that the excess lime in the bordeaux mixture rendered the calcium arsenite less soluble for a time. In an earlier discussion bordeaux was said to reduce the solubility of calcium arsenite in laboratory tests. In these tests lower solubility of the arsenite also followed the addition of lime, but upon aerating a suspension of calcium arsenite and lime for a few days the water-soluble arsenic rose to about the same level as that in a suspension of calcium arsenite without lime. On the other hand, bordeaux added to calcium arsenite not only reduced the water-soluble arsenic about as effectively as lime, but it also prevented a material increase in water-soluble arsenic during aeration of the suspension.

SUMMARY AND CONCLUSIONS

A serious blossom blight caused by *Sclerotinia laxa* is common on several stone-fruit species in California. Apricots and almonds in particular are liable to attack by the fungus, which upon entering the blossoms proceeds to invade the twigs, thus destroying much of the fruiting wood. Although this fungus sometimes attacks ripening fruit, most fruit rotting is attributable to *S. fructicola*.

As no apothecial stage of *Sclerotinia laxa* has been found in California, the entire cycle is believed to be confined to the tree. Conidia produced on blighted blossoms during the summer probably do not survive the winter. Instead, the mycelium in the blighted twigs, the invaded blossoms, and the occasional mummied fruits that hang in the tree will give rise to sporodochia during the winter. To judge from observations during three seasons, sporodochial development begins sometime in midwinter and continues for a month or more. The conidia produced on these sporodochia are the primary inoculum for blossom infection in the spring.

The present program for controlling blossom blight consists in spraying the trees with a protective spray, such as bordeaux mixture, when the blossoms emerge in the spring, and in removing the blighted hold-over twigs and fruit. Certain difficulties attend this program.

The presence in the tree of the only known hold-over stage of *Sclerotinia laxa* affords opportunities during the dormant season to reduce the survival level by spraying with eradicant fungicides. Depending upon the time of application with respect to sporodochial initiation, the eradicant fungicide would either suppress the development of these structures, or destroy them after they developed.

The experiments herein reported covered a 3-year period and consisted of approximately 161 trials of monocalcium, zinc, and sodium arsenites in apricot, almond, prune, and plum orchards located in 15 counties. The size of plots ranged from 4 trees to as high as 120. The records secured dealt with the effect of the arsenites (1) on sporodochial development, (2) on conidia present on the sporodochia when

sprays were applied, and (3) on the mycelium of the fungus in blighted hold-over twigs.

Although possessing eradicatory properties, both zinc and sodium arsenites were found to be inferior to calcium arsenite. Sodium arsenite, furthermore, proved too injurious when used in its most effective concentrations.

In many of the tests calcium arsenite, 4 pounds per 100 gallons of water, reduced sporodochial development 98 to 99 percent. Lower concentrations (3–100 and 2–100) proved somewhat less effective, but even with a concentration as low as 1–100 the eradicatory effects were noticeable. A concentration of 4–100 proved too injurious even to apricot trees, which are much more tolerant of arsenites than almonds. The concentration that appears comparatively safe to use on apricots and which possesses a high eradicant efficiency is 2–100, or possibly 2½-100.

Various materials were added to calcium arsenite in a study of their effects upon its eradicant and injurious properties. Of the materials used, petroleum-oil-emulsion alone increased eradicant properties, particularly of 2–100 and 1–100 calcium arsenite. Oil in large amounts (4 percent) tended to increase injury to apricot trees. At concentrations of 8–4-100 or even 4–2–100, bordeaux mixture and zinc-lime decreased both the eradicant and the injurious properties of the lower concentrations of calcium arsenite, but affected slightly the higher concentration (4 100). Materials such as potassium ethyl xanthate and Hydrolene No. 66 added nothing to the efficacy of calcium arsenite.

Although further tests might show that variations in the copper, lime, and arsenite components of the calcium arsenite-bordeaux mixture combination would produce a spray combining greatest safety with highest efficacy, the results thus far indicate that the safety feature can be enhanced only at the expense of eradicant efficiency. According to laboratory tests, bordeaux mixture added to calcium arsenite lowered the water-soluble arsenic content of the spray. The same was accomplished by adding lime; but, whereas lime retained soluble arsenic at a low level only so long as it remained unaerated, bordeaux continued to suppress soluble arsenic even after days of aeration.

Cultures made from blighted twigs sprayed with calcium arsenite indicate that some material entered the twigs and killed the mycelium of *Sclerotinia laxa*, thus preventing the production of sporodochia. This material is probably water-soluble arsenic, inasmuch as materials, such as bordeaux, which reduced water-soluble arsenic also reduced the eradicant effects of the weaker calcium arsenite concentrations.

Arsenites applied to the trees after sporodochia developed in large numbers were less consistent in destroying the fungus than were those applied before sporodochia developed. In 1938 both calcium and zinc arsenite killed a high percentage of conidia borne on sporodochia present when sprays were applied in February. In 1939, whereas January sprays effectively destroyed conidia then present, February sprays injured the outermost spores on the conidiophores but failed to affect those on the interior of the tightly packed mass of conidial chains. Lack of sufficient rainfall—only 0.1 inch occurred between

applications and blossoming time—to liberate soluble arsenic is believed responsible for this low toxicity.

Abundant blossom infection in the experimental orchards afforded opportunities to observe the relations between reduced sporodochial abundance (hence conidial abundance) and subsequent incidence of disease. On the whole, efficient suppression of sporodochial development brought marked disease reduction. In a few cases where treated plots were small, and hence located near untreated trees, control of the disease was not commensurate with sporodochial suppression. There is evidence that much of the infection in these plots came from conidia produced outside the treated plots and brought in by wind or by wind-borne rain.

Examples of the reduction in blossom infection obtained with calcium arsenite are as follows: 1938, 70 and 77 percent; 1939, 91 and 96 percent; 1940, 85, 91, 97, and 98 percent. The degree of disease reduction following applications of zinc and sodium arsenite paralleled their eradicant efficiencies, which, as noted earlier, were subject to fluctuations.

Control of blossom blight in plots receiving only calcium arsenite compared favorably with that in plots receiving bordeaux mixture in spring, and with that in plots receiving both the calcium arsenite and the bordeaux treatments. The eradicant treatment, however, is not expected to replace the bordeaux treatment, but to supplement it.

The injurious effects of arsenites on the tree are reported. Injury may develop from entry of arsenic into wounds, leaf scars, buds, dead twigs, or—in the almond—directly through uninjured bark. Almond trees proved so sensitive to arsenites that no effective concentration could be used on them with safety. Apricots and the plum varieties tested were more tolerant. Apricots were injured seriously only when high concentrations of arsenites were used, when applications were made soon after the trees were pruned, or when the trees were sprayed soon after the leaves fell in the autumn. Concentrations of 2–100 or 3–100 seem safe if applied in midwinter. To delay pruning until after the spray is applied avoids much of the injury through wounds. Injury through leaf scars can be minimized by postponing spraying until midwinter, at which time the leaf scars appear no longer to afford a passageway for water-soluble arsenic to enter twigs.

Although bordeaux mixture in weak concentrations (4–2–100 and 8–4–100) reduced injury to some extent, it also decreased the eradicant effect of the arsenite. Oil in large quantities increased injury, while potassium ethyl xanthate and Hydrolene No. 66 produced no change in this respect.

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A COMPARISON OF METHODS OF QUADRATTING SHORT-GRASS VEGETATION [1]

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INTRODUCTION

Permanent quadrats have long been employed in range studies where it is desired to measure the progress of changes in vegetation as a result of different systems of grazing. In some studies, description of the vegetation on such quadrats is effected by means of charts showing the position and extent of each plant; in other cases, estimates of the basal area of the vegetation or of its crown spread are made; in others the total weight or volume of each species is estimated; and in still others the plants are simply counted. The method to be used is determined largely by the characteristics of the vegetation; the method of counting would not be feasible with turf-forming species such as the short grasses, for example, but it might be useful in associations of single-stemmed plants.

In short-grass associations, descriptions of quadrats tend to be expressed in terms of area of ground cover, because of the low, matted habit of the principal species. There are many ways of determining area of ground cover—the first and second methods mentioned in the last paragraph are two of these. Until recently no critical comparisons have been made between methods. The present paper reports the results of a comparison made near Miles City, Mont., with the specific objectives of determining the degree of consistency of three methods and the causes of subjective error in each.

To the writer's knowledge, the only published comparisons of methods made prior to the present one were by Hanson and Love (5) [2] in Colorado, and by Hanson (4) in North Dakota. These studies emphasize the limitations of methods as affected by the growth habits of different prairie species, but do not treat of errors of estimate. More recently West (15) has reported on the degree of correlation between repeated estimates of the same vegetation by the density-list method as used in the Union of South Africa.

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EXPERIMENTAL PROCEDURE

METHODS TESTED

Three methods—pantograph-chart, density-list,[3] and point-analysis —were tested. For convenience the three names are shortened in the body of this paper to "chart" for pantograph-chart, "list" for density list, and "point" for point-analysis.

The chart method, illustrated in figure 1, has been described by Hill (6) and McGinnies (9). It consists essentially of reproducing a plan view of the vegetation on cross-section paper, from which areas of the various tufts can be determined later in the office.[4]

FIGURE 1.—In charting with the pantograph, the observer (right) outlines vegetation with the pointer, and the tracing is duplicated in reduced scale on the chart.

By the list method, an improved form of which has been described by Murray and Glover (10), the quadrat is divided into square decimeters, and with the aid of a small sliding frame carrying cross wires (fig. 2), the number of square centimeters of vegetation per square decimeter is estimated.

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[3] The term "density-list" is not a particularly happy one, since areas in square centimeters, not densities in percent, are listed. The apter term "area-list" has already been used (5) to denote a method by which areas of separate tufts are recorded; therefore "density-list" is adopted for the present comparison. With a listing unit of 100 cm.², as used in this comparison, the two terms come to the same thing.

[4] Records by the chart method differ from those obtained by other methods in that single shoots or very small tufts are marked by spot symbols and stolons by lines, whereas by the list and point methods such small bits of vegetation are converted directly into terms of area. In order to be able to make such a conversion from the quadrat charts, each observer was asked to submit his independent estimate of the number of spots and length of stolon which he considered equivalent to a square centimeter. The estimates were closely similar, and they have been averaged in reducing the data on the charts to an areal basis. Because the outlining of very small areas by pantograph is time consuming and seldom successful, on account of play in the instrument, tufts which in the observer's judgment were between 0.5 and 1.5 cm.² in area were drawn in by the recorder as areas of 1 cm.² These conventions probably tend to reduce somewhat the variation between observers using the chart method. In order to minimize mechanical errors the same pantograph was used for all trials on any one quadrat. Two were used, both of the type shown in figure 1, described by Pearse et al. (11).

The method of point-analysis [5] which has been employed by New Zealand workers since 1925 (*2*, *7*, *8*), and was here adapted for use on quadrats, is a record of the proportion of evenly spaced, vertically projected pin points which strike vegetation.   Thus, if 100 such points are projected downward over an area of 400 cm.[2], and 10 strike grass while 90 strike bare ground, the estimate will be that the density of the grass is 10 percent or that 40 cm.[2] of the 400 is grass.   The essential apparatus is shown in figure 3.   A series of holes in the horizontal rack enables the observer to look down along each pin, materially increasing his certainty whether a hit is made, and revealing at a glance whether a hit is impossible.
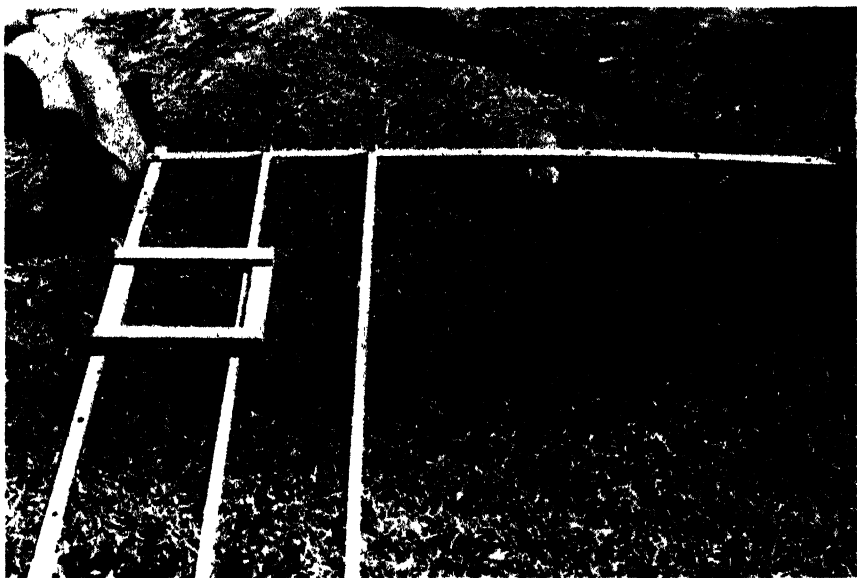
FIGURE 2.—The sliding density-list frame encloses a square decimeter divided into 25 equal parts by cross wires.   Within it an estimate is made of the number of square centimeters filled with vegetation.   In practice the straps dividing the quadrat into decimeter strips are offset to compensate for their width.

The point method gives an estimate of the amount of vegetation by a sampling process; consequently a portion of the observed variation may be attributed to sampling errors as well as to variations in judgment between observers.   Variations in results by either chart or list method, if the area estimated be constant, may be attributed almost wholly to variations in judgment.

<center>SELECTION OF QUADRATS</center>

Three quadrats of varying density were selected for the test (fig. 4).   Quadrat 1 (1 by 0.5 m.) had a sparse cover of blue grama grass (*Bouteloua gracilis*) in small, somewhat scattered, and fairly well-defined tufts.   Quadrat 2 (0.5 by 0.5 m.) supported a denser stand of mixed

[5] The point-observation-plot method, a term introduced in 1936 by Stewart and Hutchings (*14*) to describe a large-scale estimate method, has no connection with this.

grama and buffalograss (*Buchloë dactyloides*) in larger, less well-defined tufts. Quadrat 3 (0.5 by 0.5 m.) supported a high density of buffalograss forming a matted turf with no clear distinction as to tufts.

Other species on the quadrats were negligible and are omitted from the computations that follow. The two major species are so similar in growth form that they have been regarded as one in this purely quantitative test. Tufts of buffalograss lacking stolons and inflorescences are frequently confused with grama, even by trained field men.

Quadrats smaller than the customary square meter were selected because experience has shown that if 3 meter-square quadrats were to

FIGURE 3.—With the point analyzer set up at the rate of 400 points per square meter the observer (left) pushes sharpened pins down onto the quadrat one by one, and calls the hits. Then the rack is moved to the next pair of holes in the baseboards and the process is repeated.

be estimated 4 times by 5 men using 3 methods—a total of 180 estimates—the area of grass might undergo appreciable change during the time required. More rapid estimates could be made on smaller quadrats. The question naturally arises: Can estimates on a smaller quadrat be considered comparable to estimates on an entire square meter? A partial answer was found in the charts of a meter-square quadrat, made the previous year by 5 men. On this quadrat of mixed grama and buffalograss the observers' estimates varied from 2,229 to 4,678 cm.[2] Breaking each chart into quarters and expressing the area of vegetation on each quarter in percent, it is evident, as shown by table 1, that although the estimates varied widely, the observers were fairly consistent from quarter to quarter. There is reason to suppose, therefore, that if only a quarter of the square meter had been charted the relative differences between men would have been similar to those observed on the entire quadrat.
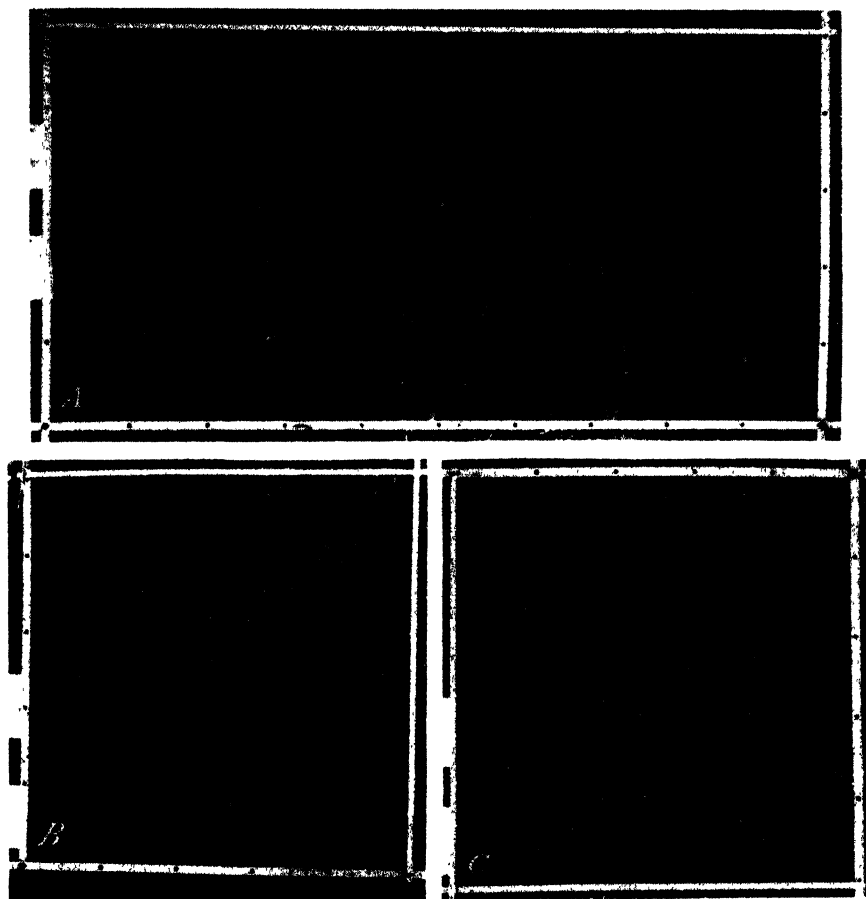
FIGURE 4.-- The three quadrats used in the test. *A*, Quadrat 1, double the size of the others, mainly blue grama. *B*, Quadrat 2, predominantly buffalograss, with some grama. The prominent tuft 12 cm. from north edge, center, is grama; buffalograss, such as the tuft to the right of this, can be distinguished by stolons. *C*, Quadrat 3, practically pure buffalograss.

TABLE 1.— *Comparison of independent observers' estimates by quarter quadrats, in percent of total estimated*

Observer	Northwest	Northeast	Southwest	Southeast	Total
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
I	26 2	23 1	24 2	26 5	100 0
J	26. 0	23. 8	24 6	25 6	100 0
K	27 5	25. 0	23. 3	24 2	100 0
L	26. 2	21 3	24 6	27 9	100 0
M	26. 9	22. 1	25 0	26. 0	100 0
Average	26 6	23 1	24 3	26 0	100 0

INSTRUCTION OF OBSERVERS

Five observers were used in the comparison. During the month prior to the test all five had been using the pantograph in regular

spring field work, having been uniformly trained and supervised in its use until the writer was convinced of their competence. If the observers were most proficient in any one, it was in this, the chart method. Most of them had little previous experience at listing and point-analyzing; but a full day (July 2) was given to preliminary trials and instructions in both methods, and it was felt that the techniques were mastered. In the course of these practice trials, standards of judgment in use of the list method were probably influenced by the results obtained in point-analyses. Inasmuch as almost any standard can be adopted for visual estimates, the later similarity in results by the two methods may be due to this fact.

It is manifestly impossible to say that these five observers constituted a representative sample of field assistants. The writer is confident that in ability they were at least not below average; they were all intelligent, well trained, and conscientious. Two were university graduates and three were students. To assume that the observed variability is due to incompetent personnel, then, is not so reasonable as to assume that some such variability may be expected in other, similarly high-grade assistants. It is noteworthy that one of the most experienced of the five men tended to make the most variable observations, and one of the least experienced the most consistent observations. The writer is led to suspect that variability in performance is closely associated with temperamental qualities.

An unavoidable limitation of the study was that the men knew they were being tested. Even though they were instructed not to let this consideration affect their work, and the writer believes they honestly tried to comply, it is scarcely to be expected that their results were wholly unaffected. A condition imposed in the trials was the rotation of recorders, so that in the first four trials no recorder accompanied an observer for more than one complete round of observations. That each man had to act both as recorder and observer was almost unavoidable. Only five men were available, and it was considered more advantageous to have five observers than to sacrifice two for full-time recorders, even though the use of five men did involve a greater risk of standardizing results. Care was taken to avoid making comparisons during the period of field work. Recorders were instructed not to compile any totals or to study the data or to comment about them in any way. It might be supposed that under some circumstances an observer's results would vary with different recorders, but such an effect is not discernible in the data from this study.

NUMBER AND NATURE OF TRIALS MADE

The purpose in having several trials on each quadrat by each man using each method was to obtain an estimate of the variability of individual observers. Circumstances which will be described prevented the attainment of this estimate of what biometricians call pure error.

In order to avoid effects of memory—which if operative would tend to reduce variability and the estimate of error— each quadrat was approached from a different side at each trial. While it is not possible to evaluate the success of this device, the observers were of the opinion that memory did not affect their work.

The original plan required each observer to make four trials of each method on each quadrat. Actually, six trials were made of the point method, the first four corresponding in time to the four trials by the chart and list methods. For the first two trials of the point method 400 points per square meter were projected. Fearing that this rate might not be sufficiently intensive to give the method a fair test, it was doubled, with the points still evenly spaced. Four trials were made at the 800-point rate, the first two corresponding in time to the last two trials by the chart and list methods. Records were kept in such a way that the pattern of points at the lower rate could be accurately segregated from these four trials. Data are therefore available for six independent trials at the lower rate as well as four at the higher rate. The additional trials complicate analysis somewhat, especially since the first two trials were at a lower rate than the last four.[6]

To have the work completed quickly was desirable, in order to minimize the change of vegetation with season. Trials were begun July 6 and completed July 23, 1936, covering an interval of 2½ weeks. It happens that the majority of observations in each trial fell inside a 4-day interval. There was no set sequence of methods, several trials of each being made on each day. Trial 1 corresponds roughly to July 6–9, trial 2 to July 10–13, trial 3 to July 14–17, and trial 4 to July 18–21. Trials 5 and 6 by the point method were made mainly July 22 and 23.

Ordinarily, no great change would have taken place in this period, but three unforeseen factors probably caused some fluctuation in density—drought, heat, and grasshoppers. The summer drought of 1936 was one of the most severe on record, and in order to have green grass on the quadrats it was necessary to water them. The countryside was suffering a grasshopper plague, and the small islands of green grass attracted grasshoppers by hundreds. Poisoning was of no avail, and finally it was necessary to place frames covered with screen or stretched burlap over the quadrats whenever they were not being worked. Intense heat, together with shade and moisture under the frames, probably resulted in forcing the grass; on the other hand, repeated handling of the vegetation, even though with great care, and a certain number of grasshoppers that somehow always managed to get under the frames, were more or less compensating factors. Judging from a series of photographs made July 9 and 21, there was not much change in the vegetation. On quadrat 1 vegetation may have been slightly denser and on quadrats 2 and 3 slightly sparser at the beginning of the trials than at the end.

This explanation is necessary in order to make clear the complexity of the factor trials. The variation between trials proved to be considerable, and it may be wondered whether it should be attributed to a progressive trend in observers' judgment or to alterations in vegetation. Apparently any great change in vegetation must be ruled out.

EXPERIMENTAL RESULTS

The source data, expressed in square centimeters of vegetation per square meter, are given in table 2. In order to study possible inter-

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[6] In the tables of source data the results of each 800-point trial are split and expressed as two separate 400-point values. The 800-point value may be obtained by averaging each pair. Unless otherwise specified, trials 1 and 2 and the corresponding halves of trials 3 and 4, printed in roman type, are the point data used in the ensuing discussion.

actions between factors, analysis of variance (*13*) of all the data was attempted; but the individual components of the highest order inter-action, used as error, proved to be so heterogeneous, apparently be-cause of the great differences in quadrat density, that this scheme had to be abandoned. Even when separate analyses were made by quadrats, there was some likelihood of heterogeneity among the error terms, apparently because the chart method gave results so different from the others. Consequently, analysis is restricted to the methods separately for each quadrat, and the opportunity to study interactions statistically is lost. In the following discussion only those data are combined which appear to be reasonably homogeneous.

DIFFERENCES BETWEEN QUADRATS

A study of averages by methods on the three quadrats reveals that, although results may differ markedly with method, on the average the methods bring out the relative areas of grass in much the same way. The ratios between quadrats 1, 2, and 3 are close to 1.00 : 1.71 : 2.85 for all three methods.

TABLE 2.—*Individual estimates of short-grass area on quadrats 1, 2, and 3, in square centimeters per square meter*

QUADRAT 1

Method and trial No.	Estimate by observer—					
	B	D	E	G	L	Average
	Square centimeters	*Square centimeters*	*Square centimeters*	*Square centimeters*	*Square centimeters*	*Square centimeters*
Pantograph-chart.						
1	1,188	1,146	1,233	1,131	1,078	1,155
2	882	941	931	1,021	1,017	958
3	826	1,083	1,058	903	804	935
4	1,120	998	960	969	933	996
Average	1,004	1,042	1,046	1,006	958	1,011
Density-list.						
1	696	606	836	772	724	727
2	710	718	746	726	700	720
3	688	676	732	612	644	670
4	706	596	734	526	578	628
Average	700	649	762	659	662	686
Point-analysis: [1]						
1	950	750	850	800	550	780
2	600	700	400	450	650	560
3	1,250	750	900	750	550	840
	750	750	600	750	800	
4	700	450	500	600	600	570
	1,100	700	600	600	550	
5	550	550	550	550	550	
	600	700	650	600	900	
6	700	550	500	550	450	
	700	650	500	750	600	
Average [2]	875	662	662	650	588	688

See footnotes at end of table.

TABLE 2. *Individual estimates of short-grass area on quadrats 1, 2, and 3, in square centimeters per square meter—* Continued

QUADRAT 2

Method and trial No	Estimate by observer—					
	B	D	E	G	L	Average
	Square centimeters	*Square centimeters*	*Square centimeters*	*Square centimeters*	*Square centimeters*	*Square centimeters*
Pantograph-chart						
1	1,603	1,362	2,752	1,815	1,761	1,859
2	1,507	1,352	1,835	1,947	1,782	1,685
3	1,682	1,632	1,603	1,686	1,448	1,610
4	1,871	1,654	1,584	1,979	1,716	1,761
Average	1,666	1,500	1,944	1,857	1,677	1,729
Density-list						
1	1,140	1,188	1,344	1,376	1,444	1,298
2	1,032	1,076	1,140	1,388	984	1,124
3	1,144	1,040	1,096	1,088	1,460	1,166
4	1,144	816	1,224	920	1,112	1,043
Average	1,115	1,030	1,201	1,193	1,250	1,158
Point-analysis [1]						
1	1,300	1,100	900	900	800	1,000
2	1,300	1,100	1,400	1,500	1,000	1,260
3	1,100	1,900	1,300	900	1,600	1,360
	1,200	*1,300*	*1,200*	*1,200*	*1,100*	
4	1,400	600	1,300	1,500	1,000	1,160
	1,600	*900*	*1,100*	*1,200*	*1,200*	
5	1,200	600	1,200	1,100	1,300	
	1,400	*990*	*600*	*1,200*	*1,300*	
6	800	400	600	1,100	500	
	700	*800*	*1,000*	*600*	*1,200*	
Average [2]	1,275	1,175	1,225	1,200	1,100	1,195

QUADRAT 3

Method and trial No	B	D	E	G	L	Average
Pantograph-chart						
1	3,259	3,275	4,596	3,957	2,208	3,477
2	2,464	2,092	3,798	3,088	2,936	2,876
3	2,385	2,960	2,994	2,712	2,672	2,745
4	3,105	2,890	3,082	3,697	2,063	2,967
Average	2,803	2,804	3,618	3,364	2,492	3,016
Density-list						
1	2,280	1,656	2,760	2,556	2,384	2,327
2	1,616	1,648	1,820	1,608	2,036	1,746
3	1,940	1,284	1,968	1,312	2,064	1,714
4	1,632	1,272	1,868	1,496	1,996	1,653
Average	1,867	1,465	2,104	1,743	2,120	1,860
Point-analysis [1]						
1	2,300	2,300	1,900	1,800	2,200	2,100
2	2,000	1,800	1,300	1,000	1,500	1,520
3	2,000	2,200	2,000	2,100	2,200	2,100
	2,400	*2,800*	*2,000*	*2,100*	*1,700*	
4	2,400	1,900	1,600	2,000	2,000	1,980
	2,700	*2,200*	*2,000*	*2,400*	*2,300*	
5	2,400	2,300	1,700	1,600	1,700	
	2,800	*1,900*	*2,200*	*2,300*	*2,200*	
6	1,900	1,400	1,200	1,900	1,800	
	2,400	*2,300*	*2,300*	*1,900*	*1,900*	
Average [2]	2,175	2,050	1,700	1,725	1,975	1,925

[1] The paired values represent separate components of the 800-points-per-square-meter rate. The upper figure, in roman type, corresponds to the 400-point rate in trials 1 and 2. Results of the 800-point rate may be obtained by averaging each pair.

[2] Average of values in roman type only, for comparison with other methods.

DIFFERENCES BETWEEN METHODS

Table 2 shows average chart values to be higher by about half than either list or point values. An examination of the individual data reveals that out of 60 cross comparisons, chart values are exceeded only four times, twice by list and twice by point values. One reason for similarity between the last two methods has already been suggested. In the data presented by Hanson and Love (*5*) in which results obtained by chart and list methods are compared, the values obtained by charting *Buchloë dactyloides* and most tufted grasses are, as here, higher. The reason is not far to seek. Few clumps of grass form a solid cover. Even when the greatest pains are taken, a pantograph can record only the larger openings, and so the numberless small ones, which aggregate a considerable area, are lumped in with the area of vegetation. But when used with care, the list and point methods do take these many small openings and marginal irregularities into account. This fact is not fully appreciated until one has worked with a point-analyzer; then he is astonished at the frequency with which a point may penetrate a dense tuft of grass without striking a single leaf.

It will be observed in table 2 that average point values are slightly higher than average list values. Is this difference significant? Table 3 analyzes the differences between individual values, and it is seen, from the probabilities given in the last line, based on *t*-tests (*3*), that observers B and D have a strong tendency toward higher point values. On the other hand, L apparently has a tendency, not quite so strong, toward lower point values. E and G show negative and positive tendencies, respectively, without marked consistency.

TABLE 3.—*Differences between individual point[1] and list values, in square centimeters per square meter*

Quadrat and trial No	Differences for observer				
	B	D	E	G	L
Quadrat 1:	*Square centimeters*	*Square centimeters*	*Square centimeters*	*Square centimeters*	*Square centimeters*
1	+254	+144	+14	+28	−174
2	−110	−18	−346	−276	−50
3	+312	+74	+18	+138	+31
4	+194	−21	−184	+74	−3
Quadrat 2					
1	+160	−88	−444	−476	−644
2	+268	+24	+260	+112	+16
3	+6	+560	+154	−38	−110
4	+356	−66	−24	+430	−12
Quadrat 3:					
1	+20	+644	−860	−756	−184
2	+384	+152	−520	−608	−536
3	+260	+1,216	+32	+788	−114
4	+918	+778	−68	+704	+154
Average	+252	+283	−164	+10	−136
P value [2]	.006	.04	.10	>.9	.07

[1] The full estimate is considered in each case; i. e., trials 3 and 4 are at the rate of 800 points per square meter.

[2] A P-value of 0.5 indicates that the departure of the average from zero is just as likely to be the result of chance as of experimental causes, a "50–50" probability; a P-value of 0.05, that the departure of the average would be the result of pure chance in 5 trials out of 100. In general, the smaller the P-value the greater the probability of significant results.

The conclusion, then, is that the slight differences between means are not the result of differences between methods so much as differ-

ences between observers; another crew with more men of "L's" temperament would presumably have produced relatively lower point values. The practical significance of this conclusion is that observers may be expected to respond differently to different methods. Biometrically speaking, there tends to be an interaction between observer and method. The existence of such an interaction is a warning against attempting to convert individual records obtained by one method into terms of some other method.

As has been noted, the point method is subject to two sources of error—personal bias (from which, if practice coincide with theory, it should be free), and errors of sampling. How big a part does each play?

Since point-analysis records were so kept that the 400 points per square meter added in the last four trials can be segregated, each of these estimates can be separated into two parts. The variations between these parts provide an estimate of sampling error, as opposed to error derived from the observer-trial interaction. From separate analyses of variance, including a break-down of quadrat 1 into north and south halves, each 0.5 by 0.5 m., table 4 is derived. In general the paired variances are strikingly similar. The variation (12 degrees of freedom) designated "interaction" is neither greater nor less, consistently, than sampling variation (20 degrees of freedom), and one is led to conclude that the errors of personal bias by the point method are of the same order of magnitude as those inevitable in sampling.

TABLE 4.- *Sampling variances as compared with interaction variances of the point method, and probabilities of significant differences in magnitude*

Quadrat	Interaction variance	Sampling variance	P
Quadrat 1			
Entire	16, 219	21, 750	.69
North half	63, 542	44, 500	.23
South half	33, 792	40, 500	62
Quadrat 2	104, 417	64, 750	17
Quadrat 3	52, 750	123, 750	.93

A practical problem that confronts the field worker with the point method is to know what number of point projections he should use in order to attain a desired degree of accuracy. If the points were distributed at random rather than in a rigid pattern, he would expect sampling accuracy to vary in inverse proportion to the square root of the sample size. Since in practice he is dealing with a rigid rather than a random pattern, in which each projection is not completely independent of every other, what may he expect? May he utilize sampling theory at all?

If sampling theory is applicable, we should expect the variation between 800-point values in the last four trials to be smaller than that between 400-point values by the inverse of the square root of 2, or 1.41. The last two columns in table 5, with four out of six values greater than 1.41 and two smaller, give no good reason for concluding the theory other than applicable in a broad rule-of-thumb way. Hence, within the limits of this empirical test, it would seem that after some preliminary determinations one might arrive at any

accuracy desired, by altering the frequency of point projection in accordance with theory.

TABLE 5.—*Effect of doubling point-analysis rate: Standard deviations from sampling by 800 and 400 points per square meter*

Quadrat No	s_{800}	s_{400} (even)	s_{400} (odd)	$\dfrac{s_{400}\ (\text{even})}{s_{800}}$	$\dfrac{s_{400}\ (\text{odd})}{s_{800}}$
1	90.1	122 7	141.6	1.36	1 57
2	228.5	363.2	242 7	1.59	1.06
3	162.4	248.2	275.4	1.53	1 70

DIFFERENCES BETWEEN OBSERVERS

Some differences between the results of different observers are to be expected; yet, with every possible precaution taken, it is surprising how great such differences may be. For example, quadrat 3 as charted July 14 by B had 2,385 cm.^{2} of grass; but as charted on the same day by D it had 2,960 cm.^{2} or 575 cm.^{2} more. Three days later, B charted an area of 3,105 cm.^{2} on the same quadrat and D an area of 2,890 cm.^{2}, this time 215 cm.^{2} less! This example illustrates how observers tend to report results that are not only different but in many instances inconsistent.

At the same time, some rather fixed differences between observers are apparent, for, while there are many exceptions, E tended to be the highest and L the lowest man with the chart method, E the highest and D the lowest with the list method, and B the highest and L the lowest with the point method.

A summary of the significance of differences between observers, by quadrats and methods, is given in table 6, in which probabilities for the respective analyses of variance are computed with the help of Pearson's Tables of the Incomplete Beta-Function (*12*). Differences between observers are most clearly marked on quadrat 3, and least clearly marked on quadrat 2. A satisfactory explanation is lacking, except that quadrat 3, being the most difficult quadrat of the three, probably gave most opportunity for the exercise of personal judgment.

TABLE 6.—*Probabilities that differences between average areas reported by different observers and at different trials are due merely to chance*

OBSERVERS

Quadrat No	Pantograph-chart method	Density-list method	Point-analysis method [1]		
			400(4)	800(4)	400(6)
1	65	07	.14	.07	.05
2	.28	33	96	57	66
3	.04	006	.03	.01	.006

TRIALS

	Pantograph-chart method	Density-list method	Point-analysis method [1]		
1	.009	.05	.02	.02	.002
2	59	.11	.43	.02	.04
3	.15	.001	.003	.09	.003

[1] In the column headed 400(4), the third and fourth trials at 400 points per square meter are included with the first 2; the column headed 800(4) relates to the last 4 trials at 800 points per square meter; the column headed 400(6) includes all 6 trials at the rate of 400 points per square meter.

The data in table 7 will serve to illustrate some of the curious quirks of observers. They are selected because on two occasions each of the observers used all methods on the same quadrat the same day. Given these conditions, the same sort of variation could probably be demonstrated between any other observers. E's values by charting and listing are markedly lower on July 20 than on July 6 and his point values are almost the same. D's results, on other days than E's but similarly near the beginning and end of the work, are quite different; although he, like E, arrives at less area by listing on the later date, his areas by charting and point-analyzing are markedly higher.

DIFFERENCES BETWEEN TRIALS

On quadrats 1 and 3 results from the first trial average higher than results from later trials, and it is this difference which is reflected in the low *P* values for these quadrats in table 6. The likelihood of a major vegetation change, which has already been discussed, does not seem great enough to account for so sharp a difference. The writer is convinced, from taking part in the work as well as scrutinizing the results, that after the first two days, with the establishment of a work routine, there was a general swing among observers toward conservatism in estimate. This swing was by no means unanimous, but it was strong enough to produce much of the observed difference.

TABLE 7.— *Individual estimates of area of buffalograss on quadrat 3, in square centimeters per square meter, by two men, each having made all three estimates on the date given*

Observer and date	Pantograph-chart method	Density-list method	Point-analysis method
	Square centimeters	*Square centimeters*	*Square centimeters*
Observer E			
July 6	4, 596	2, 760	1, 900
20	3, 082	1, 868	1, 800
Observer D			
July 9	2, 092	1, 648	1 800
17	2, 890	1, 272	2, 500

RELATIVE VALUE OF THE METHODS

Since in this comparison there is no absolute standard with which observed values may be compared, it is impossible to determine which method of the three comes nearest to giving the true values. The point method, because it is the most mechanical, might be expected to reflect vegetation area without bias, and perhaps it comes nearest of the three methods to doing so; but as the probabilities in table 6 indicate, there were consistent differences between observers using the point method, so that this expectation is not fully realized.

IN CONSISTENCY

Since we cannot know which method is most accurate absolutely, the next best thing is to know which is most consistent within itself. An appropriate measure of variability, the inverse of consistency, is the square root of the variance, or the estimated standard deviation

based upon the observed values. Estimates of standard deviations in the present test, since they are based on only 20 values (30 in the next to last column in table 8), are themselves subject to considerable error and can be considered only as rough approximations.

TABLE 8.—*Standard deviations, in square centimeters of vegetation per square meter, and as percents of the respective means*

Quadrat No.	Pantograph-chart method	Density-list method	Point-analysis method [1]		
			400(4)	400(6)	800(4)
	Square centi-meters	*Square centi-meters*	*Square centi-meters*	*Square centi-meters*	*Square centi-meters*
1	90	54	151	125	90
2	292	152	345	307	228
3	491	216	210	234	162
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
1	8.9	7.9	21.9	19.5	13.6
2	16.9	13.1	28.9	28.2	21.2
3	16.3	11.6	10.9	12.4	7.8

[1] In the column headed 400 (4), the third and fourth trials at 400 points per square meter are included with the first 2; the column headed 800 (4) relates to the last 4 trials at 800 points per square meter; the column headed 400 (6) includes all 6 trials at the rate of 400 points per square meter.

In discussing averages it was pointed out that densities of the quadrats, although expressed on a different level by the chart from that by the others, are reflected similarly by all methods. A pattern is discernible in table 8 which shows variability to be more complex than this. The list method has, in the main, the smallest standard deviation; that is, of the three methods it tends to be the most consistent. Variability of both chart and list methods increases from quadrat 1 to 3, but variability of the point method is greatest for quadrat 2. The standard deviations expressed as percents of the means in the lower part of table 8 do not follow these same trends, which makes it seem unlikely that increases in variability of the chart and list methods from quadrat 1 to 3 are associated altogether with increasing density. Probably the low standard deviations of chart and list methods on quadrat 1 are associated with the predominance of small, compact tufts of grass on that quadrat, estimation of which tends to be standardized. Such definite tufts were lacking on quadrat 3.

Variability with increasing density increases somewhat more markedly by the chart than by the list method. This is probably associated with the decreasing definiteness of tuft outline from quadrat 1 to 3, making charting increasingly more difficult.

The tendency toward low standard deviations by the point method on quadrat 3 is probably associated with the fact that vegetation and bare spots were well distributed over the quadrat. The distribution on quadrat 1 was better than on quadrat 2 from the viewpoint of the point analyst, since the tufts, in general, were scattered, small, and open; whereas those on quadrat 2 tended to be larger and more compact. Thus, if a certain point hit a leaf on quadrat 2 there was more chance of its next neighbor striking a leaf also (differences in density aside) than there would have been on quadrat 1; and the same is true for a strike on bare ground. In other words, there was least likelihood of correlation between successive projections on quadrat 3

and most likelihood on quadrat 2; and the greater the correlation, the greater the likelihood of variability under the conditions of this test.[7]

IN RAPIDITY

Rapidity in a method is almost as important as a minimum of random error. The less time required for each quadrat, the greater the number of quadrats that can be examined, and the more reliable the resulting average will be. Moreover, the more rapidly the examinations can be made, the less the data will be influenced by changes in the vegetation with season. This is an especially important consideration where, as in the short-grass region, field work may be seriously curtailed by summer drought.

TABLE 9.—*Average field time per trial and average office time required for compilation* [1]

Method	Quadrat 1			Quadrat 2			Quadrat 3		
	Field	Office	Total	Field	Office	Total	Field	Office	Total
	Man-minutes	*Man-minutes*	*Man-minutes*	*Man-minutes*	*Man-minutes*	*Man-minutes*	*Man-minutes*	*Man-minutes*	*Man-minutes*
Pantograph-chart	191	120	311	160	104	264	239	175	414
Density-list	62	5	67	45	5	50	51	5	56
Point-analysis									
400 per meter [2]	58	3	61	37	3	40	48	3	51
800 per meter [2]	72	3	75	48	3	51	58	3	61

[1] Quadrat 1 = 0.5 m.²; quadrats 2 and 3 = 0.25 m.² each.

In the field a record was made of time consumed by each trial, and in the office, of the time required for its compilation. The averages, given in table 9, show that the chart method is 4 to 8 times as costly in total time as the other two.[8] In point of field time, a unit area of quadrat 3, which had about three times as much vegetation as quadrat 1, took about two and a half times as long to chart—that is to say,

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[7] The explanations in this paragraph were tested with the aid of the field records. Inasmuch as this test may be of interest in application of the point-analysis method for studying the distribution of vegetation, it will be described

Considering only the results of projections along the axis of the rack, occurrences of two hits together, two misses together, or a hit and a miss together were tallied. The frequencies of these occurrences were then compared with the frequencies which would have occurred if hits and misses had been distributed completely at random. Random distribution is given by weighing the expansion of $(p+q)$ [1] by the proportion of the number of hits (p) and misses (q, where $q = 1 - p$) to the total number of trials. The term p^2S (where S is the total number) gives the number of expected hits, q^2S the number of expected misses, and $2pqS$ the number of expected hits and misses. The last four point-analyses (800 points per square meter) on each quadrat were used for the test. The averages of 20 deviations, observed minus expected, and their standard errors are as follows.

Quadrat	Miss and miss	Hit and hit	Miss and hit
1	−0.738±0.454	+*0.890±0.283*	−0.153±0.634
2	+*.910± .388*	+*1.085± .570*	−*1 998± .695*
3	−.901± 574	−.174± .421	+*1.074± .932*

The probability that these departures may be due solely to chance may be found by using a table of t such as given by Fisher (*3*). It will be found that P for the values in italics is less than 0 05, and these differences may therefore be thought of as being consistent enough to be real. For quadrat 1 there is a marked tendency for hits to fall together, but this tendency does not seem to affect the random occurrence of misses together, or of misses and hits together. This indicates numerous small, scattered tufts. For quadrat 2 there is a marked deficiency of misses and hits, and a tendency for hits to fall together and for misses to fall together. This indicates fairly large tufts and bare spaces; the deficiency probably arises from this cause and also from a tendency of the grass to be concentrated around the edges of this quadrat (A single hit in the middle of the quadrat gives rise to two occurrences, a miss-hit and a hit-miss, but a hit by the point at the end of the rack can result in only half this much detail). Since the departures for quadrat 3 are well within the limits of expectancy, it is to be concluded that the distribution of grass on quadrat 3 is not proved other than random.

[8] Operation of the pantograph requires two men, but in the list and point methods only one need be used. It should be noted that the trials and time estimates used in this test are on a two-man basis, and hence allow for the list and point methods a considerably greater number of man-minutes than are needed in actual field practice.

working time is roughly proportionate to density—whereas by the other two methods the dense quadrat took only a little more than half again as long as the sparse one. Compilation of the list and point records took in every case less, usually much less, than one-twentieth the time required for chart compilation.

IN CONSISTENCY AND RAPIDITY COMBINED

In the preceding discussion it has been shown that the methods have differing degrees of variability and require different amounts of time, both of which are costs. Assuming the familiar relation to hold, that the squared standard error of the mean equals the squared standard deviation divided by the number of observations, we may determine the relative efficiencies (3), or inverse relative costs, of the three methods. Table 10 gives the efficiency components and their product in relation to the variability and time for the list method.

Strictly on the basis of time, the chart method is much less efficient than the others, and on the basis of variability, it is much less efficient than the list method. In its relation to the point method, the variability-efficiency of the chart method shows an interesting change from quadrat 1 to 3. On the scattered vegetation of quadrat 1, it is greater than that of the lower point-analysis rate and about equal to that of the higher rate; on the grouped vegetation of quadrat 2 it is lower than on quadrat 1, although still greater than the efficiency of the lower point-analysis rate; and on the dense vegetation of quadrat 3, it is far lower than the efficiency of the point method at either rate.

TABLE 10.—*Combination of the cost factors, variability (s^2), and time (T), relative to those of the list method; all terms expressed inversely as "efficiency"*

Method	Quadrat 1			Quadrat 2			Quadrat 3		
	$\frac{1}{s^2}$	$\frac{1}{T}$	$\frac{1}{s^2T}$	$\frac{1}{s^2}$	$\frac{1}{T}$	$\frac{1}{s^2T}$	$\frac{1}{s^2}$	$\frac{1}{T}$	$\frac{1}{s^2T}$
Pantograph chart	36 9	21 5	7 9	27 0	18 9	5.1	19 5	13.5	2 6
Density-list	100 0	100 0	100 0	100.0	100 0	100 0	100 0	100 0	100 0
Point-analysis (400)	13 0	109 8	14 3	19 3	125 0	24.1	105 8	109 8	116 2
Point-analysis (800)	36 5	89 3	32 6	44 1	98 0	43 2	177.5	91 8	162 9

When the two factors are combined, the chart method is seen to have between one-twelfth and one-fortieth the efficiency of the list method, its relative efficiency becoming less as the vegetation increases in density and complexity. Depending upon sampling intensity, the relative efficiency of the point method varies here from one-seventh to more than half again as much as that of the list method, and, in contrast to the relative efficiency of the chart method, increases with increasing vegetation density. The efficiency of the point method can be considerably increased on vegetation of low density by increasing the number of points; that is, the decrease in variability from doubling the number of points more than makes up for the increase in time cost. On vegetation of higher density this increase in efficiency is progressively less marked.

ADAPTABILITY TO VARIED USE

The merits of these methods should be examined also in the light of the principal uses to be made of them. If comparison is to be made of the effects of known grazing treatments upon short-grass range over a period of years, including a record of the amount and kind of vegetation resulting under each treatment, and if data are desired on such ecological problems as conditions which govern seedling establishment or competition between individual plants of different species, periodical mapping may be necessary, and the chart method is the appropriate choice. If, on the other hand, the principal object is to know accurately magnitude of changes, rather than detail of processes involved in them, a more rapid, more accurate means, such as the list method, is desirable.

The chart method, besides providing a kind of pictorial history, showing the location and shape of each plant in relation to all the others, permits correction of many misidentifications. Chart data, however, are not always expressed in consistent terms, so that if one wishes to speak of spot symbols in terms of area, he must use an arbitrary conversion factor.

The list method, while it proved most efficient in this study, may not prove so in general application unless its use is carefully controlled. Estimates without a sound standard of reference are likely to vary between wide, and even wild, extremes. Hence it is necessary, in applying the list method, to provide some means of standardization. In the present test standardization was achieved by all observers working together in preliminary trials, and their concept of plant density was probably strongly influenced by the use of the point method, which they considered to be a more objective method than the others.

The principle of the point method was tested during the winter prior to the present test on quadrat charts on which the areas of outlined vegetation had been determined. About 400 intersections of regularly spaced lines were adopted as points, and it was found that the percentage of points falling within outlines of the principal species agreed closely with the area percent of those species. Under necessity to prepare a quick summary of uncompiled chart areas later, the writer applied the same method and rapidly made compilations sufficiently accurate for the immediate purpose. Abell (*1*) has recently described an application of the same principle to determine the areas of irregular figures on maps.

Another possible use of the point method, not touched upon in this paper, is the direct determination of volume by the total number of hits, that is, hits at all distances above the ground (*7, 8*).

The variability of any method is probably at a minimum on an unobstructed quadrat such as the ones used in the present test. On many short-grass ranges, inclusion of cactus and sagebrush is unavoidable if the quadrats are intended to be truly representative. These plants interfere with operation of the pantograph and often require that the density-list frame be held at some distance from the ground. Tests made in the summer of 1937 showed that when the density-list frame is raised 7 inches off the ground, the variability of repeated estimates is markedly increased. Provided that sufficiently

long sliding pins are obtainable, the point method is probably superior to the others under such conditions.

The three methods are capable of improvement in varying degree. A more rigidly constructed pantograph would reduce some though not the most important errors of the chart method. Doubtless, greatest improvements are possible in the point method, by using better sighting devices to minimize the number of doubtful hits, by using longer pins which slide more smoothly, with finer harder points, and by reducing the awkwardness and weight of the apparatus. The apparatus as used in this comparison was bulkier than that used by New Zealand workers, but the sharpened uprights of the instrument used by Levy and Madden (8), when repeatedly driven into the soil in an arid climate, probably would affect the quadrat environment seriously. The even simpler device diagramed by Fenton (2) has a similar objection and seems less adapted to precise work.

SUMMARY AND CONCLUSIONS

Three methods of quadratting short-grass vegetation—the pantograph-chart, density-list, and point-analysis methods (referred to as "chart," "list," and "point")—were tested on three typical short-grass quadrats, of low, intermediate, and high density. Five trained observers used each method four times on each quadrat, except that the point method was used six times at two different intensities. A 2-week period was required for the comparison, during which, it was concluded, no appreciable changes in the vegetation took place.

On an average, the methods reflected the marked differences in grass area between quadrats similarly, although with differing absolute values.

With all methods, consistent differences between observers were most evident on the quadrat with grass of highest density and most matted habit.

One observer, it was found, may record consistently greater areas by one method than by another, and another observer may record lesser areas by the first method and greater areas by the second; thus there may exist an interaction between observer and method.

In contrast to consistent differences between the results of different observers, inconsistencies, sometimes of considerable magnitude, appeared within the work of a given observer, in spite of elaborate precautions in training and standardization.

Areas by the chart method tended to be 50 percent greater than areas by the other methods, and the chart method proved to be less consistent than the list method on all quadrats and more consistent only than the lower-rate point method on the low- and medium-density quadrats. Since it required much more time than the list and point methods, its net efficiency, within the limits tested, varied from one-half to less than one-fiftieth of theirs.

Areas by the list method tended to be similar in magnitude to those by the point method, which may be due to preliminary standardization practice. The list method gave more consistent results than the others (except for the point method on the high-density quadrat). It required about the same amount of time as the point method. Its net efficiency was much higher than that of either of the other methods

on the low- and intermediate-density quadrats, but lower than that of the point method on the high-density quadrat.

The two components of variation in the point method, sampling error and personal bias, were of about the same order of magnitude in this test. Although the points are not projected at random, one may arrive fairly close to any desired accuracy within the limits tested by altering the number of projections in accordance with sampling theory. As a corollary, since the method is a rapid one, its efficiency can be increased by increasing the number of projections. The benefit of such an increase was most marked with vegetation of low density.

In view of the results of this comparison, it is suggested that for estimating area of short-grass vegetation on intensively studied, permanent quadrats which are intended to sample the effects of grazing treatments, the density-list method, carefully standardized, be applied. The point-analysis method, pending its further development, may well be used for training and standardizing observers in the density-list method. The pantograph-chart method should be reserved for those studies in which the greatest need is a detailed graphic record of the vegetation.

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THE PROCESS OF AMINO ACID FORMATION FROM SUGARS IN ASPERGILLUS NIGER [1]

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INTRODUCTION

A great deal of work has been done on the carbon requirements of the fungi (*13*),[3] and many of their metabolic products have been identified (*10*). Notwithstanding the vast quantity of data accumulated, little is known concerning the fundamentals of the carbon nutrition of these fungi. Though much has been learned regarding the respiratory and fermentative degradation of the sugars, the manner of their employment for synthesis of the protein that is necessary for growth is quite unknown.

The distinction made between the respiratory and fermentative fragmentation of the sugars, on the one hand, and the synthesis of protein for growth, on the other, is considered to be of great importance. *Aspergillus niger* Van Tiegh., for example, can be grown in such a manner that little increment in weight takes place and about 90 percent of the sugar is converted to citric acid. The writer (*16*), on the other hand, has frequently obtained growth equivalent to 52 percent of the sugar supplied. It is evident, therefore, that the relative quantities of carbon assimilate used in these phases of metabolism vary in an extreme manner with environmental conditions.

Observations on the growth of *Aspergillus niger* with glycerol furnished the incentive for the investigations here described. The possibility of improved results with the trace elements by the substitution of glycerol for sucrose had been noted (*15*). It was found, however, that yields with different samples of glycerol ranged from 10 to 400 mg. Though it was found possible to increase yields almost to maximum by the addition of traces of various organic substances, the effective materials were not considered such as were likely to be present as impurities. Moreover, the results on trace elements were rendered poorer, if anything, through the use of such organic compounds, as compared to the high yields obtained with certain samples of reagent glycerol. It seemed advisable, therefore, to make a general study of the carbon assimilation of the fungus in order to arrive at some indication of the type of substances that might be responsible for the observed differences.

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Unfortunately, an adequate study of carbon assimilation required the use of many compounds that were unobtainable or obtainable only at great expense because of the small commercial demand. In most instances no one could be found to undertake the preparation of the small quantities needed, either because of the expense involved or because methods of preparation were unknown. It was therefore only through the generosity of investigators in the chemistry of the sugars that the present investigations were made possible. Many gave freely of their own supplies, which were frequently very limited.

Despite such collaboration, it was impossible to carry the work beyond a preliminary stage. Nevertheless, it is believed that enough has been accomplished to indicate clearly the probable course of carbon assimilation involved in the formation of amino acids by the fungus. However, circumstances have required the termination of the studies, and it is doubtful whether they can be resumed.

METHODS AND MATERIALS

The cultural methods employed were similar to those previously described (*14*). *Aspergillus niger* was grown in 200-cc. pyrex Erlenmeyer flasks in all work not involving a direct study of trace elements. Otherwise all laboratory ware used was of transparent fused quartz. Water used in the preparation of nutrient solutions was distilled through a metal still and then through a quartz still.

The standard nutrient solution was prepared by dissolving in a liter of water 50 gm. of sucrose, 1.90 gm. of ammonium nitrate, 0.35 gm. of dipotassium phosphate, and 0.25 gm. of magnesium sulfate (7 H_2O). Iron, zinc, copper, manganese, molybdenum, and gallium were added in concentrations of 0.30, 0.30, 0.075, 0.075, 0.02, and 0.02 mg. per liter, respectively. The fungus was grown on 50-cc. portions of the solution for 4 days at a temperature of 35° C.

The fungus was harvested by filtering the cultures with sintered glass crucibles of No. 3 porosity. The yields were determined after the fungus had been dried overnight at 103° to 105° C.

Substitutions of other carbon compounds [4] for sucrose were effected by omitting the sucrose and adding the compound directly to the culture. It was necessary to check on the acidity that developed in the nutrient solution through the use of compounds other than sucrose and to add sufficient calcium carbonate to bring the solution within a range of pH 5 to 7 when necessary.

[4] The following compounds were obtained through the kindness of the investigators mentioned:
D-Gulose.½CaCl₂.H₂O, D-α-glucoheptose, D-α-galaheptose, and D-xylonic-γ-lactone, from Dr. Horace S. Isbell, U. S. Bureau of Standards, Washington, D. C.
D-Glyceraldehyde and L-glyceraldehyde, from Prof. E. E. F. Baer and Prof. Hermann O. L. Fischer, Banting Institute, Toronto, Canada.
L-Glucose (preparation of H. O. L. Fischer), from Dr. Harry Rudnick, Banting Institute, Toronto, Canada.
D-Sorbose (preparation of Emil Fischer), from Prof. Hermann O. L. Fischer, Banting Institute, Toronto, Canada.
L-Allose, L-altrose, and L-ribose, from Prof. Fred L. Humoller, Loyola University, School of Medicine, Chicago, Ill.
D-Mannoketoheptose (preparation of F. B. La Forge), from Dr. Elias Yanovsky, Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture, Washington, D. C.
Kojic acid, calcium 2-keto-D-gluconate, calcium 5-keto-D-gluconate, calcium *d*-lactate, and calcium D-gluconate, from Dr. G. E. Ward, Northern Regional Research Laboratory, Bureau of Agricultural Chemistry and Engineering, U. S. Department of Agriculture, Peoria, Ill.
Acetone-D-threose and acetone-D-xyloketose, from Prof. T. Reichstein, Pharmazeutische Anstalt der Universitat, Basel, Switzerland.
L-Tagatose and L-galactose, from Prof. Koichi Iwadare, Chemical Institute, Imperial University, Tokyo, Japan.
D-Ribonic acid lactone and D-arabonic acid, from Dr. Joseph Rosin, Merck & Co., Rahway, N. J.
L-Xylose, from Dr. Hardy W. Larson, Biochemical Laboratory, Metropolitan Life Insurance Co., New York, N. Y.

CARBON UTILIZATION FACTOR

Before attempting to estimate the assimilability of carbon compounds, an experiment was carried out with sucrose (table 1) to ascertain the effect of concentration upon the unit it was planned to use as a basis for comparison. This unit, or "carbon utilization factor," as it will be called, is the value obtained by dividing the yield in grams by the number of grams of carbon present in the substrate; i. e., it is the yield in grams (weighed after drying at 103°–105° C.) per gram of carbon supply.

The carbon utilization factor was found to vary somewhat with the concentration at which the sucrose was employed. It would seem that respiratory and fermentative reactions tended to be emphasized at low concentrations, since values for grams of yield per gram of carbon decreased somewhat. A maximum was reached at concentrations ranging from 50 to 60 percent of optimum. The value again decreased at still higher concentrations because of a presumed greater and greater proportion of unutilized sucrose.

TABLE 1.—*Assimilation of sucrose by Aspergillus niger during 4 days of growth at 35° C.*

Sucrose per culture	Carbon per culture	Yield of fungus	Yield per gram of carbon supply	Relative yield per gram of carbon supply	Sporulation [1]
Grams	*Grams*	*Grams*	*Grams*		
0.075	0.0316	0.0333	1.054	0.898	4
.150	.0632	.0541	[2](.856)	[2](.729)	6
.250	.1053	.1132	1.075	.916	8
.500	.2105	.2333	1.108	.944	8
.750	.3158	.3660	1.159	.987	8
1.000	.4210	.4866	1.156	.985	8
1.250	.5263	.6102	1.159	.987	8
1.500	.6315	.7419	[3] 1.174	1.000	8
1.750	.7368	.8542	1.159	.987	8
2.000	.8420	.9427	1.120	.954	8
2.250	.9473	1.0063	1.062	.905	8
2.500	1.0525	1.0113	.961	.819	8

[1] Rated from 0 (sterile) to 10 (completely covered with spores).
[2] Yield inconsistent with the other values.
[3] The carbon utilization factor of sucrose is therefore equivalent to 1.17.

For accurate comparison of the assimilability of carbon compounds, it was necessary to take the concentration factor into consideration. This was accomplished by dividing the carbon utilization factor for any compound by the relative yield per gram of carbon obtained with sucrose at an equivalent carbon concentration. The term "carbon utilization factor," as employed in this paper, will refer always to such values as have been so corrected for concentration. It was found convenient to plot the relative yields per gram of carbon obtained with sucrose against carbon supply, and thus obtain the intermediate values by inspection.

ASSIMILABILITY OF CARBON COMPOUNDS

Growth and sporulation of the fungus were quite poor with the majority of one-, two-, and three-carbon chain compounds investigated (table 2). Of the two-carbon compounds tried, glycolic and glyoxylic acid gave best results as nutrients. Nielsen and Hartelius

(8) ·have suggested that these, together with glyceric acid, may function with *Aspergillus* as accessory growth factors. Of the three-carbon compounds, dihydroxyacetone and glyceric acid showed the highest carbon utilization factors. The D– form of glyceraldehyde gave better results than did the L– form. Absence of growth on pyruvic acid had not been anticipated. Lack of growth with the acetal of glycolaldehyde does not necessarily imply that glycolaldehyde would not support growth. The uniform absence of growth with the esters that were investigated may indicate that the strain of fungus used had a low hydrolytic capacity.

TABLE 2.—*Assimilation of organic carbon compounds by Aspergillus niger during 4 days of growth at 35° C.*

ONE- AND TWO-CARBON COMPOUNDS

Source of carbon supply		Yield of fungus	Carbon utilization factor	Sporulation [1]
Compound	Quantity per culture			
	Grams	*Gram*		
Acetaldehyde	1.0	0.0021	0.0	1, br
Ethylene glycol	1.0	0	0	0
Glycolic acid	1.0	.0136	.04	0
Glyoxylic acid	1.0	.0358	.11	1
Oxalic acid	1.0	0	0	0
Glyoxal, polymerized	1.0	0	0	0
Calcium formate	1.0	0	0	0
Ethyl alcohol	1.0	0	0	0
Calcium acetate	1.0	.0033	.01	0
Glycolaldehyde diethylacetal	1.0	0	0	0

THREE-CARBON COMPOUNDS

D-Glyceraldehyde	2.5	0 0939	0 11	1
L-Glyceraldehyde	.9433	0284	.08	6
Dihydroxyacetone	.6452	0828	.33	8
Glycidol	1 0	0	0	0
Propylene glycol	1.0	0	0	0
Pyruvic acid	1.0	0	0	0
Trimethylene glycol	1 0	.0006	0	0
Glyceric acid	.5	.0415	25	2
►Glycerol	2.5	.0087	.01	1
Allyl alcohol	1.0	0	0	0
Malonic acid	1.0	.0041	.01	1
Acrolein	1.0	0	0	0
Sodium acrylate	1.0	0	0	0
Barium mesoxalate	1.0	0	0	0
Sodium glycerophosphate	1.0	0	0	0
Calcium d-lactate	1.0	.0190	.08	2
Propionaldehyde	1.0	0	0	0
Propylene oxide	1.0	[2] +	0	0

FOUR-CARBON COMPOUNDS

D-Threose [3]	0.25	0.0011	0.01	0
1, 3-Butylene glycol	1.0	0	0	0
2, 3-Butylene glycol	1.0	0	0	0
Succinic acid	1.0	0	0	0
dl-Tartaric acid	1.0	.1081	.39	10
Mesotartaric acid	1.0	.0058	.02	1
l-Malic acid	1.0	.0915	.26	10
dl-Malic acid	1.0	.0121	.03	2
Fumaric acid	1.0	.0634	.20	10
d-Tartaric acid	1.0	.0968	.31	10
Ethyl oxalacetate (sodium salt)	1.0	0	0	0
Ethyl acetoacetate	1.0	0	0	0
Sodium dihydroxytartrate	.5	0	0	0

:See footnotes at end of table.

TABLE 2.—*Assimilation of organic carbon compounds by Aspergillus niger during 4 days of growth at 35° C.*—Continued

FIVE-CARBON COMPOUNDS

Source of carbon supply		Yield of fungus	Carbon utilization factor	Sporulation [1]
Compound	Quantity per culture			
	Grams	*Gram*		
D-Lyxose	1.0	0 0002	0	0
D-Xylose	2.0	.8602	1.09	10
L-Xylose	.5	0062	03	1
D-Arabinose	2.0	0	0	0
L-Arabinose	2.0	.2051	.27	6
D-Ribose	.25	+	0	0
L-Ribose	25	.0052	.05	0
D-Xyloketose [4]	.25	.0005	.01	0
Glutaric acid	1 0	.0043	.01	0
Levulinic acid	1 0	.0040	.01	0
Itaconic acid	.5	0024	.01	0
Ethyl acetopyruvate	1.0	0	0	0
Furfural	1.0	0	0	0
Ethyl acetone dicarboxylate	1.0	0	0	0
β-Methyl-D-xyloside	1.0	0	0	0
D-Ribonic lactone	.25	.0025	.03	0
D-Arabonic acid (glassy)	.5	+	0	0
D-Xylonic-γ-lactone	.25	.0071	.08	1

SIX-CARBON COMPOUNDS

D-Galactose	2 5	+	0.07	
L-Galactose	2	+	0	0
D-Gulose [½ CaCl₂.H₂O]	5079	0	0	0
D-Mannose	2.5		1.46	10
D-Glucose	2.5		1.43	10
L-Glucose	.25	0	0	0
L-Altrose	.25	+	0	0
L-Allose	.25	0	0	0
D-Fructose	2.5		1.38	10
L-Sorbose	2.5		1.26	10
D-Sorbose	.0843	+	0	0
L-Tagatose	.10	0035	.10	0
D-Digitoxose	.988	0	0	0
L-Fucose	.9788	0	0	0
L-Rhamnose (H₂O)	1 0	.1215	.31	4
L-Ascorbic acid	.25	.0069	.08	0
i-Inositol	.5	+	0	0
Calcium 2-keto-D-gluconate	1.0	.2011	.67	0
Calcium 2-keto-D-gluconate (heated)	1.0	3120	1.04	10
Calcium 5-keto-D-gluconate	1.0	.0252	.08	1
Calcium D-gluconate	1.0	.0317	.10	2
Kojic acid	1.0	.0001	0	0
Mucic acid	1 0	<.1024	<.30	1
α-Methyl-D-glucoside	5	.0001	0	0
Calcium citrate	1 0	.0019	.01	0
α-Methyl-D-mannoside	.5	0	0	0
Citraconic anhydride	1 0	0	0	0
D-Isoascorbic acid [5]	1 0	0991	.24	10
Calcium D-glucuronate	1.0	.2062	.62	10
D-Gluconic lactone	1.0	.0058	.01	0

SEVEN-CARBON COMPOUNDS

D-α-Galaheptose	0.5	0	0	0
D-α-Glucoheptose	.5	+	0	0
D-Mannoketoheptose	5	0 0063	0.03	0
D-Glucoascorbic acid [5]	1.00	.1577	.39	10

[1] Rated from 0 (sterile) to 10 (completely covered with spores). Color other than black is indicated by br (brown).
[2] Plus sign (+) indicates germination.
[3] Acetone-D-threose was used after decomposition by acetic acid (*11*).
[4] Acetone-D-xyloketose was used after decomposition by acetic acid (*11*).
[5] Substrate became alkaline.

Attention is called to the fact that each oxidation product of glycerol (glyceraldehyde, dihydroxyacetone, and glyceric acid) investigated gave a much better yield than did glycerol. It is evident, therefore, that partial oxidation of reagent glycerol may be a factor in the greater yields often obtained. This cannot be the entire explanation, however, since such substances would be apt to be present only in small concentrations. The acceleration in growth reported to take place on addition of sodium iron chlorophyllin and hemoglobin (*15*) may be associated with an increased oxidative capacity. Increases in yield by amino acids (*14, 15*) have been shown to be due to the use of a poor carbon source. These would seem to be instances of empirical results whose significance could not be conjectured prior to systematic study.

Responses to four-carbon chain compounds (table 2) were on the whole no better than to one-, two-, and three-carbon derivatives. Superior results were obtained with *dl*-tartaric acid, *d*-tartaric acid, *l*-malic acid, and fumaric acid. Since *dl*-tartaric acid has the configuration of LD-threose, and *d*-tartaric acid that of L-threose, it would seem that the D- form of threose should prove more assimilable than the L- form. D-Threose, however, gave a carbon utilization factor of only 0.01.

Of the seven pentoses investigated, only D-xylose gave a yield approaching that with sucrose (table 2). The reason is not clear for the somewhat better results obtained with L-arabinose and L-ribose than with their D- forms.

The responses given by the fungus with D-xylose, D-lyxose, and D-xyloketose have great significance for an understanding of the process of pentose assimilation. It is well known that the reason assumed for the high and practically equivalent assimilation of D-glucose, D-mannose, and D-fructose is their possession of a common enol; they are epimers. The three pentoses enumerated are also epimers but, unlike the epimeric hexoses just mentioned, give widely different results on assimilation.

Table 2 also gives a summary of the results on assimilability obtained with six-carbon compounds. Only 9 of the 16 aldohexoses could be obtained. Of these, only D-glucose and D-mannose were completely assimilable. Of 5 ketohexoses tried (8 are known), only 2 proved efficacious as sources of carbon; these were D-fructose and L-sorbose. Moderate growth was given by L-rhamnose, D-isoascorbic acid, 2-keto-D-gluconic acid, and D-glucuronic acid.

Oxidation of the aldehyde group in glucose to form gluconic acid had destroyed its nutritive properties. Further oxidation at the 2-carbon atom brought about partial recovery in assimilability, whereas oxidation at the 5-carbon atom did not. Ohle and Berend (*9*) have reported that D-arabinose and D-arabonic acid are formed from 2-keto-D-gluconic acid by heating in solution. These products accounted for less than half the 2-keto-D-gluconic acid present,

however. In view of the unavailability of D-arabinose and D-arabonic acid for growth of *Aspergillus*, it is of interest to note that two periods of heating (20 minutes at 100° C.) increased the assimilability of the 2-keto acid considerably (table 2).

Only with D-glucoascorbic acid, of the seven-carbon compounds (table 2), did the fungus show any appreciable growth. This substance and D-isoascorbic acid gave exceptional results in that the substrates became strongly alkaline through growth of the fungus. L-Ascorbic acid had the lowest carbon utilization factor; D-isoascorbic acid gave a higher value, and D-glucoascorbic acid a still higher value (table 2). In other words, the D- configuration was superior to the L- configuration. The greater usefulness of D-glucoascorbic acid is perhaps due to its partial decomposition at the 1, 2 double bond to form D-glucose or D-mannitol.

CHEMICAL STRUCTURE AND ASSIMILABILITY OF MONOSES

It seems clear from the data of table 2 that the rate of carbon utilization by *Aspergillus* was quite poor with all compounds having less than a five-carbon atom chain. Of the five-carbon compounds, only D-xylose approached maximum efficacy in assimilation. Maximum values for utilization of six-carbon compounds were given by D-glucose, D-mannose, D-fructose, and L-sorbose. That is, only one pentose and four hexoses were found to possess full carbon assimilability.

The basis for the situation just described is attributed to the necessity for preliminary decomposition and condensation reactions when compounds with less than a five-carbon chain are employed. This interpretation is increased in probability by the greater degree of growth given by known degradation products of the sugars. These are dihydroxyacetone, glyceraldehyde, glyceric acid, glycolic acid, and glyoxylic acid. These decomposition reactions probably are reversible to some degree or at least are better fitted for necessary preliminary condensations.

A general idea of the configurations (5) and yields of the aldoses and ketoses can be obtained from the following schematic arrangement. The positions of the hydroxyl groups in only the D- aldoses are given, since those of the L- forms can be obtained by substituting minus signs for plus signs, and vice versa. The zeros in the ketose configurational notation represent the keto (=CO) group.

ALDOSES

Glyceraldehyde
D = +
D = 0.11; L = 0.08

Erythrose
D = +
D = ?; L = ?

Threose
D = - +
D = 0.01; L = ?

Ribose
D = ++++
D = 0.00; L = 0.05

Arabinose
D = - + +
D = 0.00; L = 0.27

Xylose
D = + - +
D = 1.09; L = 0.03

Lyxose
D = - +
D = 0.00; L = ?

Allose
D = +++; +++ L = 0.00

Altrose
D = ++ +
D = ?; L = 0.00

Glucose
D = - + +
D = 1.43; L = 0.00

Mannose
D = - + +
D = 1.46; L = ?

Gulose
D = + + +
D = 0.00; L = ?

Idose
D = + -
D = ?; L = ?

Galactose
D = - +
D = 0.07; L = 0.00

Talose
D = - - +
D = ?; L = ?

	L-Glucose	D-Glucose
(1)	HCO	HCO
(2)	HOCH +	HCOH +
(3)	HCOH +	HOCH -
(4)	HOCH -	HCOH +
(5)	HOCH -	HCOH +
(6)	H₂COH	H₂COH

	L-Fructose
(1)	H₂COH
(2)	CO 0
(3)	HOCH +
(4)	HOCH -
(5)	HOCH -
(6)	H₂COH

	D-Fructose
(1)	H₂COH
(2)	CO
(3)	HOCH -
(4)	HOCH -
(5)	HCOH +
(6)	H₂COH

KETOSES

Dihydroxyacetone
0
(0.33)

D-Erythrulose
0 +
(?)

L-Erythrulose
0 -
(?)

D-Araboketose
0 + +
(?)

D-Xyloketose
0 - - +
(0.01)

L-Xyloketose
0 + -
(?)

L-Araboketose
0 - -
(?)

D-Tagatose
0 - - +
(?)

D-Sorbose
0 + - +
(0.00)

D-Psicose
0 + + +
(?)

D-Fructose
0 - - +
(1.38)

L-Sorbose
0 - -
(1.26)

L-Tagatose
0 + +
(0.10)

L-Fructose
0 + -
(?)

L-Psicose
0 - -
(?)

A check of structure against yield in the above scheme will disclose that every one of the sugars capable of giving maximum growth has a minimum chain of five carbon atoms and an L-3-carbon atom and a D-4-carbon atom. Monoses deficient in these particulars failed to sustain the maximum rate of growth. These are not the only requirements, since D-lyxose and D-xyloketose have these configurational prerequisites but cannot give maximum growth. The same situation probably exists with L-idose and L-gulose, the epimers of L-sorbose. Monoses having the reverse configuration, as L-gulose in comparison with D-glucose, and L-xylose in comparison with D-xylose, are apparently unable to support growth. The fungus was deficient, therefore, in the ability to transform such compounds to assimilable forms by simultaneous reduction and oxidation of opposite terminal carbon atoms. This process with D-glucose might account for the formation of L-ascorbic acid and L-sorbose in green plants.

These statements may be readily verified through examination of the structural formulas and carbon utilization factors in the following arrangement of monoses. All pentoses and hexoses in which the 3- and 4-carbon atoms have opposite configurations have been included and are indicated by asterisks. Each group of three consists of epimers that are readily convertible into one another by means of a common enol. This relation is indicated by arrows. Response of *Aspergillus* to D-glucose, D-fructose, and D-mannose is similar to that of yeast and would seem to confirm the interpretation that the enol common to these three sugars participates in assimilation. That this assumption is incorrect should prove clear when L-idose and L-gulose become available. *Aspergillus* will probably be found unable to employ these unnatural or synthetic sugars. Gortner (*2*) points out that though some yeasts can slowly ferment D-galactose, they cannot attack D-tagatose and D-talose, its epimers.

HCO
HCOH
HOCH
HCOH
HOCH
H₂COH
L-Idose
(?)

H₂COH
CO
HOCH
HCOH
HOCH
H₂COH
L-Sorbose
(1.26)

HCO
HOCH
HOCH
HCOH
HOCH
H₂COH
L-Gulose
(?)

(1)
(2)
(3*)
(4*)
(5)
(6)

HCO
HOCH
HOCH
HCOH
HCOH
H₂COH
D-Mannose
(1.46)

H₂COH
CO
HOCH
HCOH
HCOH
H₂COH
D-Fructose
(1.38)

HCO
HCOH
HOCH
HCOH
HCOH
H₂COH
D-Glucose
(1.43)

HCOH
HCOH
HCOH
HOCH
HCOH
H₂COH
D-Idose
(?)

H₂COH
HCOH
HOCH
HOCH
HCOH
H₂COH
D-Sorbose
(0.00)

HCOH
HCOH
HOCH
HCOH
HCOH
H₂COH
D-Gulose
(0.00)

(1)
(2)
(3*)
(4*)
(5)
(6)

HCO
HCOH
HCOH
HOCH
HOCH
H₂COH
L-Mannose
(?)

H₂COH
CO
HCOH
HOCH
HOCH
H₂COH
L-Fructose
(?)

HCO
HCOH
HOCH
HCOH
HCOH
H₂COH
L-Glucose
(0.00)

HCO
HCOH
HOCH
HOCH
H₂COH
L-Lyxose
(?)

H₂COH
CO
HCOH
HOCH
H₂COH
L-Xyloketose
(?)

HCO
HCOH
HCOH
HOCH
H₂COH
L-Xylose
(0.03)

(1)
(2)
(3*)
(4*)
(5)

HCO
HOCH
HCOH
HCOH
H₂COH
D-Lyxose
(0.00)

H₂COH
CO
HOCH
HCOH
H₂COH
D-Xyloketose
(0.01)

HCO
HCOH
HOCH
HCOH
H₂COH
D-Xylose
(1.09)

The behavior of D-xylose and its epimers affords a similar proof that enolization is not concerned in assimilation. D-xylose gave excellent growth; D-xyloketose permitted germination; and D-lyxose gave no germination.

The differences indicated in behavior of epimers are presumably associated with requirements for initial attack. The chains shown as straight, for convenience, do not exactly conform to that structure, but even on the basis of furanose and pyranose rings, no explanation of these differences in behavior is obvious. The pyranose structures of D-glucose and its epimers are identical with those of D-xylose and its epimers, except that they contain a "—CHnOH—" instead of a₂ "—H" on their fifth carbon atoms (4). These modifications result in very different effects.

The use of *Aspergillus* instead of yeast should prove of great aid in the study of these phenomena, since the number of epimeric groups, and therefore the number of derivatives suitable for study, is thereby greatly increased. Yeast, as a matter of fact, cannot be used, since the only group of epimers it can attack shows practically no differences in behavior of the individual sugars. Studies with compounds having an L-3- and L-4-carbon atom that are unassimilable (D-xyloketose, D-lyxose) or are readily transformed to such theoretically (L-gulose, L-idose) appear desirable for an understanding of the requirements for initial attack.

Instances showing the importance of a 1-, 2-, 5-, or 6-carbon atom in attack and assimilation of the hexoses have been found. Helferich and Bigelow (3) stated that yeast is unable to ferment 5-keto-D-glucose, whereas Micheel and Horn (6) found that 5-keto-D-fructose was only about half as effective as glucose. Fischer (1) found that yeast cannot ferment D-glucosone (2-keto-D-glucose). With *Aspergillus* the introduction of an amino group at the 2-carbon atom (D-glucosamine) brings the carbon utilization factor down to 0.03. Moreover, as shown in the following arrangement, the oxidation of D-glucose to D-gluconic acid renders the sugar practically unassimilable, though with further oxidation to 2-keto-D-gluconic acid it becomes about half as effective as D-glucose. Oxidation of the 5-carbon atom of D-gluconic acid does not increase assimilability.

OCH   OCOH   OCOH   OCOH
| | | |
HCOH   HCOH   CO   HCOH
| | | |
HOCH   HOCH   HOCH   HOCH
| | | *or* |
HCOH → HCOH → HCOH → HCOH
| | | |
HCOH   HCOH   HCOH   CO
| | | |
H₂COH   H₂COH   H₂COH   H₂COH

 D-Glucose D-Gluconic acid 2-Keto-D-gluconic 5-Keto-D-gluconic
  (1.43)   (0.10)   acid (0.67)   acid (0.08)
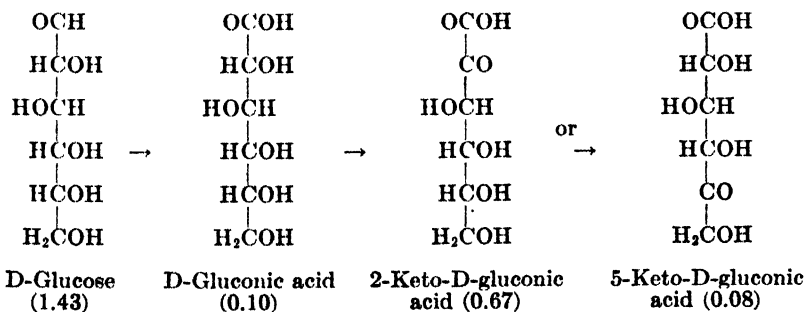
While oxidation of D-glucose at the 1-carbon atom to D-gluconic acid renders it practically unassimilable, oxidation at the 6-carbon atom (to D-glucuronic acid) only halves assimilability (C. U. F.⁵ = 0.62). Another instance, in which oxidation at the 6-carbon atom has the opposite effect, is shown by comparing L-sorburonic acid

⁵ Carbon utilization factor.

(i. e. 5-keto-D-gluconic acid) and L-sorbose, as shown in the following arrangement. It is necessary to rotate the structural formula of the former through 180° in the plane of the page.

H₂COH	H₂COH	OCOH

$$\begin{array}{ccc}
\text{H}_2\text{COH} & \text{H}_2\text{COH} & \text{OCOH} \\
\text{CO} & \text{CO} & \text{HCOH} \\
\text{HOCH} & \text{HOCH} & \text{HOCH} \\
\text{HCOH} & \text{HCOH} & \text{HCOH} \\
\text{HOCH} & \text{HOCH} & \text{CO} \\
\text{H}_2\text{COH} & \text{OCOH} & \text{H}_2\text{COH}
\end{array}$$

| L-Sorbose (1.26) | L-Sorburonic acid (0.08) | 5-Keto-D-gluconic acid (0.08) |
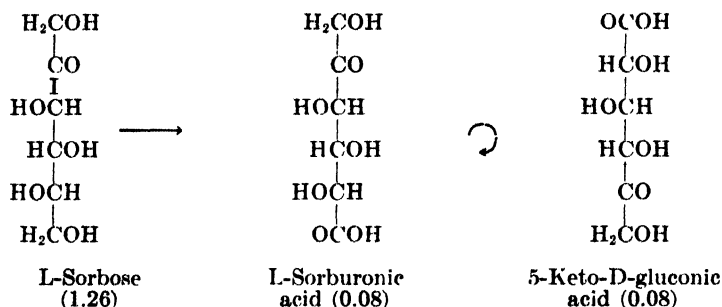
The absence of results with a large number of oxidation products of D-glucose makes interpretation of their behavior difficult and somewhat speculative. The important distinction to be kept in mind in this connection is that even minute modifications in structure usually render the sugar completely unassimilable. An alteration in structure that does not do so probably approaches that of the true intermediate.

It would seem likely as a result of these data that α-keto-D-glucuronic acid or a derivative is the intermediate that undergoes decarboxylation. In this connection, investigation should be made of assimilability of the 2- and 5-keto-D-glucosaccharic acids. These have not yet been prepared, however. Tamiya (*17*) found D-glucosaccharic acid (saccharic) 30- to 60-percent assimilable by *Aspergillus oryzae*. Introduction of an α-keto group should have the effect of further increasing assimilability, as occurred with D-gluconic acid.

Assimilation of hexose, would seem on this basis to comprise a highly complicated series of enzymatic reactions. These include oxidation to an α-keto acid and decarboxylation. Whether the other terminal carbon atom is oxidized to carboxyl during the first stage or whether this oxidation takes place in the pentose derivative that is formed remains to be determined. Its presence in the amino acids definitely indicates its formation, however. Moreover, the constitution of the amino acids requires removal of hydroxyl from pentose or hexose in their formation, accomplished possibly through dehydration and reduction.

CONVERSION OF SUGARS TO AMINO ACIDS

Any hypothesis concerning the transformation in *Aspergillus* of sugars to amino acids must provide explanations for the following facts:

(1) Use of ammonia in formation of the amino group in amino acids (*14*).

(2) Direct synthesis of primary amino acids: Proline, glutamic acid, ornithine (*16*).

(3) Presence of levo amino groups alpha to carboxyl in amino acids.

(4) Presence of beta hydroxyl groups in all hydroxyamino acids, but no gamma or delta group.

(5) Presence of dextro hydroxyl groups beta to carboxyl in amino acids.

(6) Relative unassimilability of compounds with less than a five-carbon atom chain.

(7) Necessity for L-3- and D-4-carbon atoms in assimilable sugars.

(8) Unassimilability of certain sugars by certain organisms.
(9) Unassimilability of D-xyloketose, D-lyxose, and probably L-idose and L-gulose.
(10) Relatively better assimilability of glyceraldehyde, glyceric acid, dihydroxyacetone, glycolic acid, and glyoxylic acid.

A series of chemical reactions meeting the requirements named is as follows:

H_2COH
CO
$HOCH$
$HCOH$
$HCOH$
H_2COH

D-Fructose (1.38)
D-Glucose (1.43)
D-Mannose (1.46)

H_2COH (1)
CH_2 (2)
$HOCH$ (3*)
$HCOH$ (4*)
CO (5)
$OCOH$ (6)

H_2COH
CO
$HOCH$
$HCOH$
$HOCH$
H_2COH

L-Sorbose (1.26)

CH_3 (2)
$HOCH$ (3*)
$HCNH_2$ (4*)
$OCOH$ (5)

l-Threonine [*d*(−)]

HCO
$HCOH$
$HOCH$
$HCOH$
H_2COH

D-Xylose (1.09)

HCO (1)
CH_2 (2)
$HOCH$ (3*)
$HCOH$ (4*)
$OCOH$ (5)

(To illustrate configurations in hydroxy acids only)

$OCOH$ (1)
CH_2 (2)
$HOCH$ (3*)
$HCNH_2$ (4*)
$OCOH$ (5)

l-Hydroxyglutamic acid

NH₃   NH₃   NH₃

$OCOH$ (1)
CH_2 (2)
CH_2 (3*)
$HCNH_2$ (4*)
$OCOH$ (5)

l-Glutamic acid (0.22)

CH_2
CH_2
CH_2
$HCNH$
$OCOH$

l-Proline (0.22)

(1)
(2)
(3*)
(4*)
(5)

⇌

H_2CNH_2 (1)
CH_2 (2)
CH_2 (3*)
$HCNH_2$ (4*)
$OCOH$ (5)

l-Ornithine (0.10)

CH_2 (1)
CH_2 (2)
$HOCH$ (3*)
$HCNH$ (4*)
$OCOH$ (5)

l-Hydroxyproline

$H_2CNH—C=NH$
CH_2 NH_2
CH_2
$HCNH_2$
$OCOH$

l-Arginine (0.10)

$OCOH$ (1)
CH_2 (2)
$HCNH_2$ (3*)
$OCOH$ (4*)
      (5)

l-Aspartic acid (0.22)

A detailed discussion of each point is unnecessary. It should be understood, however, that it is intended only to indicate the general paths followed in these transformations. No evidence was obtained for participation of the intermediates shown nor for their number. A degree of certainty exists only as concerns the initial and end products.

Threonine, serine, hydroxyglutamic acid, and hydroxyvaline are beta hydroxy acids of natural occurrence, whereas hydroxyproline is either the beta or gamma hydroxy acid. Only in the case of threonine is the configuration of the hydroxyl group known. Except possibly hydroxyglutamic acid, none is thought to be formed directly from the sugars.

The direct formation of glutamic acid, ornithine, proline, and possibly aspartic acid from a sugar derivative with a five-carbon atom chain is postulated because of the results of a previous investigation (*16*). The results are discussed and amplified in the next section. Schoenheimer and Ratner (*12*) found, through the use of isotopic nitrogen, that in mice conversion of ornithine to proline, arginine, and glutamic acid is an automatic reaction.

The dihydroxy-aldehyde-acid represented as a possible 5-carbon atom intermediate compound is the α-hydroxy acid-aldehyde corresponding to β-hydroxyglutamic acid. This amino acid has been found to occur in nature. Because of the difficulties associated with its isolation, very little of this material is available, and none could be obtained for use in these experiments.

EFFICIENCY OF ANABOLITES AS SUBSTITUTES FOR MONOSES

It has been shown that sucrose has a carbon utilization factor of 1.17; D-mannose, 1.46; D-glucose, 1.43; D-fructose, 1.38; D-sorbose, 1.26; and D-xylose, 1.09. Moreover, though glutamic acid and proline gave factors of about 0.22 and ornithine 0.10, a mixture of these three amino acids reached a factor of 0.83. This is 58 percent of the glucose value and may indicate the proportion of carbon passing through an amino acid stage during growth.

Table 3 contains the results of miscellaneous additional experiments. Though pyruvic acid by itself did not even permit germination to take place, it appeared, when added to an amino acid mixture, to be slightly more effective than sucrose as a source of carbon. Substitution of *l*-malic acid for *l*-aspartic acid when *l*-proline and *d*-glutamic acid were also present did not lead to diminished utilization. Use of a 5 : 9 mixture of *l*-proline and *l*-aspartic acid gave a factor of 0.63, while a similar mixture of *l*-proline and *l*-malic acid reached a factor of 1.12. In evaluation of these results it must be borne in mind that interconversion of aspartic and glutamic acids probably occurs as readily as in green plants (*7*). Deamination of aspartic acid produces malic acid.

Evidently a mixture of amino acids and carbon compounds is possible that is fully as effective as any sugar. Since requirements for this mixture are specific in nature, the probability exists that duplication of the important initial products of sugar decomposition is being accomplished to a greater or less extent. Full equivalence would possibly be reached by use of a mixture of hydroxy acids—*l*-α-hydroxyglutaric acid, *l*-α,δ-dihydroxy-valeric acid—corresponding to deaminated glutamic acid, proline, and ornithine. No source of supply could be located for these hydroxy acids, so experiments with them were not possible.

TABLE 3.—*Carbon utilization factors for mixtures of compounds obtained with Aspergillus niger after 4 days of growth at 35° C.*

Source of carbon		Mixtures of carbon compounds in cultures on which *Aspergillus niger* was grown																		
l-Aspartic acid	gm	0.25		0.25	0.25	0.25	0.25	0.45	0.45	0.45	0.45	0.45	0.45	0.25	0.25	0.25	0.25		0.25	0.25	0.25
d-Glutamic acid	gm	.25	0.25		.25	.25	.25		.25		.25	.25		.25	.25	.25	.25		.25	.25	.25
l-Proline	gm	.25	.25	.25		.25	.25	.25				.25	.25	.25	.25	.25	.25	0.25	.25		.25
d-Arginine hydrochloride	gm					.25				.25											
d-Ornithine dihydrochloride	gm						.25		.25	.25											
dl-Alanine	gm												.25	.25							
l-Leucine	gm														.25						
l-Hydroxyproline	gm															.25					
l-Malic acid	gm																	.45	.25		
Fumaric acid	gm																			.25	
Pyruvic acid	gm																.25				
Sucrose	gm																				.25
Carbon utilization factor (sucrose=1.17)		0.66	0.45	0.49	0.17	0.79	0.82	0.63	0.81	0.82	0.77	0.74	0.83	0.46	0.70	0.40	0.89	1.12	0.83	0.83	0.80

TRACE-ELEMENT REQUIREMENTS WITH THE MONOSES

A résumé of the trace-element requirements of the fungus with the monoses is given in table 4. Nutrient solutions purified by treatment with calcium carbonate were employed in making these tests (*14*). The data for the different sugars are quite uniform, with one exception: D-xylose gave an exceptionally high yield in cultures from which the iron had been removed. The reason for this response was not investigated. The data would definitely refute the claim that has been made in the past that zinc is necessary only for the utilization of sucrose.

DISCUSSION

Investigations into the biological conversion by plants of sugar to amino acids have been inconclusive in the past, it is believed, because of the choice of unsuitable organisms for study. Green plants furnish unfavorable material because the quantity and nature of the sugar to be employed cannot be controlled in a normal environment. Bacteria are unsuitable because of very low yields as contrasted to quantity of dissimilation products. Fungi, however, are uniquely fitted for such studies, if, like *Aspergillus*, they are of unspecialized nutrition.

The use of cultivated yeasts, to the practical exclusion of other fungi, has been an unfortunate choice. Not only are these organisms practically confined to the use of D-glucose and its epimers, but they are also characterized by a great predominance of fermentative reactions. It is possible in this connection that use of D-galactose by "trained" yeasts depends on its complete break-down and the recombination of the fragments. *Aspergillus niger*, however, assimilates D-xylose and L-sorbose in addition, and can convert a large proportion of these sugars to cellular material under conditions where fermentation is practically suppressed. *Aspergillus*, moreover, would seem to differ less from green plants than the yeasts, since D-xylose (wood sugar) and L-sorbose (L-ascorbic acid configuration) are also participants in the metabolism of the green plants.

Assimilation of hexoses would seem to depend primarily on the presence of an L-3-carbon and a D-4-carbon configuration. Only hexoses meeting this requirement support growth since they meet the configurational requirements of the amino acids. Initial attack may occur at the 1- or 6-carbon atom, a derivative of an α-keto acid probably being formed. Decarboxylation results in the formation of a compound having a chain of five carbon atoms. Probably L-gulose, like L-xylose, cannot be modified by the fungus to meet its requirements, though simultaneous oxidation and reduction at opposite ends of the carbon chain would accomplish this change.

Oxidation and decarboxylation reactions probably account for specificity in initial attack of the hexoses. *Aspergillus* utilizes all hexoses having a satisfactory 3-,4-carbon atom configuration, except probably L-gulose and L-idose, whereas yeasts cannot attack these or L-sorbose. This distinction in capacity between these two kinds of fungi would indicate the probability that break-down of D-glucose and L-sorbose is dependent upon different enzymatic systems. The differences shown by D-xylose, D-lyxose, and D-xyloketose with *Aspergillus* would also indicate a very delicate adjustment in enzymatic attack. The only distinction between these compounds is that they possess a D-2-carbon, an L-2-carbon, and a 2-keto-carbon

TABLE 4.—*A comparison of trace-element requirements of Aspergillus niger with different sugars when grown at 35° C. for 4 days in solutions purified with calcium carbonate*

Element omitted	D-Glucose Yield per 2.5 gm. (Milligrams)	Relative yield (Per cent)	Acidity at harvest (pH)	Starch in mycelium	Sporulation	D-Fructose Yield per 2.5 gm. (Milligrams)	Relative yield (Per cent)	Acidity at harvest (pH)	Starch in mycelium	Sporulation	L-Sorbose Yield per 2.5 gm. (Milligrams)	Relative yield (Per cent)	Acidity at harvest (pH)	Starch in mycelium	Sporulation	D-Mannose Yield per 2.5 gm. (Milligrams)	Relative yield (Per cent)	Acidity at harvest (pH)	Starch in mycelium	Sporulation	D-Xylose Yield per 2.5 gm. (Milligrams)	Relative yield (Per cent)	Acidity at harvest (pH)	Starch in mycelium	Sporulation
None	1,152.9	100.00	3.36	3		1,131.3	100.00	4.00	E	10	1,059.8	100.00	2.57	0	10	1,211.2	100.00	2.99	2	10	1,053.0	100.00	5.38	E	10
Fe	4.5	0.39	3.22	0		17.9	1.58	3.68	0	0	8	2.01	3.36	0	2	13.5	1.11	3.63	0	4	1,005.3	95.46	2.30	0	6
Zn	21.6	1.88	2.87	0		65.6	5.80	2.59	0	1	25.8	2.43	3.50	0	8	21.2	1.75	3.28	0	2	39.3	3.73	2.78	0	2
Cu	860.0	74.60	2.48	2		w940.5	83.14	3.08	1	4,w	w1,049.8	99.05	2.58	*5(1)	4,w	899.5	74.27	2.73	*5(1)	8	1,021.0	96.95	3.86	0	10.t
Mn	685.4	59.45	1.62	*5(3)		1,016.9	89.88	1.62	*3	2,v	664.7	62.68	1.67	0	4	774.5	63.95	1.57	0	4	1,057.3	100.41	4.49	E	10
Mo	1,137.2	98.65	2.27	3		986.5	88.08	1.77	3	6	985.7	93.95	2.23	0	10	1,106.4	91.34	2.29	0	6	1,018.1	96.68	1.77	*5(5)	4
Ga	1,119.7	97.13	3.42	3		1,130.4	99.92	3.86	3	10	1,059.4	98.08	2.54	0	10	1,181.4	97.54	3.49	2	10	1,051.6	99.86	4.48	E	10
Max [3]	1,197.2					1,164.1					1,060.8					1,228.5					1,068.4				
C.U. [4]	47.89					46.56					42.43					49.14					42.74				
pH [5]	7.17					6.97					6.25					7.12					6.68				

[1] Starch is rated on a scale of 0 (absent) to 5 (profuse), an asterisk (*) indicating immediate formation of a blue color on addition of N/20 iodine solution. Values in parentheses represent comparable data for the substrates E=erythrodextrin. When spores were not black, color is indicated by t (tan), y (yellow), and w (white).
[2] Rated from 0 (sterile) to 10 (completely covered with spores)
[3] Maximum individual yield.
[4] Coefficient of utilization, or yield per 100 gm. of sucrose.
[5] Initial acidity of the nutrient solution.

atom, respectively. It seems possible that enzymatic reduction at the 2-carbon atom is involved with the pentoses, though this may be accompanied by oxidation at other points. The D-xylose and the L-sorbose enzyme systems would seem to be absent in the yeasts.

While amino acid configurational requirements for levo-α-amino and dextro-β-hydroxyl groups sharply limit the list of assimilable monoses, a mixture of primary amino acids was almost as effective in maintaining growth. Neither the ornithine, glutamic acid, nor proline comprising this mixture possessed an asymmetric carbon atom (hydroxyl) in the 3 position. The explanation for this seeming contradiction is not obvious in the light of our present knowledge. A simple initial procedure that may prove of aid for investigation of this difference might be based on the requirements for a mixture fully equivalent to the D-glucose.

Absence of vigorous growth on glycerol would seem to depend on an oxidative incapacity of the fungus. Glyceraldehyde, dihydroxyacetone, glyceric acid, glyoxylic acid, and glycolic acid—all probable oxidation products of glycerol—gave better growth than did glycerol. The tartaric acids behaved similarly as compared to D-threose. The condensation mechanism whereby short-chain inactive molecules are combined to form optically active compounds is the opposite of the ordinary process of nutrition. This is true in the preliminary stages at least. There is some evidence that dissimilative break-down of hexose to triose phosphate is reversible. An interesting phase of comparative studies of growth on sucrose and on glycerol concerns itself with possible changes in quantitative requirements for trace elements in the two types.

SUMMARY

Carbon utilization factors (grams of yield per gram of carbon supply) for about 120 compounds, obtained with *Aspergillus niger* Van Tiegh. grown for 4 days at 35° C., were as follows: D-glucose, 1.43; D-mannose, 1.46; D-fructose, 1.38; L-sorbose, 1.26; and D-xylose, 1.09. Other compounds were poor sources of carbon supply. Enolization was not a factor in sugar utilization. All pentoses and hexoses having an L-3-carbon atom and a D-4-carbon atom were assimilable, except the epimers of D-xylose and probably L-sorbose. Preliminary oxidation to α-keto acid derivatives and decarboxylation appeared necessary for assimilation of hexoses.

The hypothesis was proposed that the aldehyde derivative of *l*-α,β-dihydroxy *n*-valeric acid served as precursor for amino acid formation. D-4-carbon of the monoses became *l*-α-amino carbon in the amino acids, and L-3-carbon remained unchanged to become *d*-β-hydroxy in *d*(-)threonine, and possibly in hydroxyglutamic acid and hydroxyproline also. The primary amino acids formed were probably proline, glutamic acid, and ornithine—all α,δ-derivatives of *n*-valeric acid— since mixtures of these attained a carbon utilization factor of 0.83 as compared to 1.17 with sucrose. Evidence was obtained that the carbon utilization factor for amino acid mixtures was increased by substitution of the corresponding hydroxy acids.

Growth requirements for trace elements with assimilable sugars showed little difference, except that cultures on D-xylose from which iron had apparently been removed by treatment with calcium carbonate gave exceptionally high yields. Trace-element requirements with monoses were quite similar to those with sucrose.

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THE RELATION OF ZINC TO SEED PRODUCTION [1]

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INTRODUCTION

The importance of zinc for the complete development of many agricultural plants has been demonstrated by numerous workers in recent years. The subject has gained additional interest from the discovery that certain soils do not supply the amounts of zinc necessary for every crop.

REVIEW OF LITERATURE

The zinc in many noncultivated forest soils, according to Hibbard (5),[3] tends to accumulate in zones less than 5 inches below the surface as a result of the deposition of leaves and twigs and the rapid fixation of zinc when liberated by the processes of decay.

Sommer and Lipman (12) and Chandler (3) presented conclusive evidence that zinc is a necessary element for the growth of a variety of agricultural plants. Chandler's observation that zinc deficiency is responsible for the imperfect development of fruits and nuts of various orchard trees is of especial interest in connection with the subject of this paper. He found that blossoms borne on trees suffering from zinc deficiency failed to set a normal crop of fruit. The yield was therefore defective in quantity as well as quality. An application of a small amount of zinc during the winter resulted, however, in the production of healthy foliage and a normal crop of fruit on trees that had formerly manifested symptoms of serious zinc deficiency.

It is intended in this paper to present the results of experiments to determine the relation of zinc to seed production in peas (*Pisum sativum* L.), beans (*Phaseolus vulgaris* L.), and dwarf milo (*Sorghum vulgare* Pers.). The relation of the microelements to seed production is a matter of very great importance, since many agricultural crops are grown almost exclusively for the seed produced. It is obviously difficult to differentiate the effects of an element on the vegetative and reproductive activities of the plant because of the close interrelationship of the two processes and their dependency upon each other. Nevertheless, a beginning has been made, and the results of the experiments may be noted briefly.

The function of zinc in the reproductive processes of plants was first studied in the mold fungi. Although the process of conidial formation in fungi is obviously quite different from reproduction in the angiosperms, it will yet be profitable to notice a few recent studies on the phenomenon.

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Butkewitsch and Orlov (*2*) reported that zinc sulfate accelerated mycelial growth and increased the efficiency of the utilization of sugar by the fungus, although a wide variation in concentration of zinc did not cause marked differences in the growth of the mycelium. Steinberg (*13*) and Gollmick (*4*) found that the presence of a small amount of iron, zinc, copper, and manganese is essential for the normal growth and sporulation of *Aspergillus niger*. Lockwood, Ward, and May (*7*) reported that the addition to the culture medium of 10 mg. per liter of zinc as zinc sulfate increased the weight of mycelium, the consumption of glucose, and the production of dextrolactic acid by *Rhizopus oryzae*. Waksman and Foster (*15*) arrived at somewhat different conclusions. They reported that zinc stimulates growth and respiration but does not affect the accumulation of lactic acid in the culture. Lockwood (*6*) found that spore germination of *R. oryzae* was favored by the presence of zinc, and further reported that in a concentration of 0.01 mg. of zinc per liter of solution, germination and sporulation were accelerated, but the yields of lactic acid were inversely proportional to the concentration of zinc in the culture solution.

Sommer's experiments demonstrated (*11*) that zinc was highly important for the reproductive processes of Windsor beans. The growth of the plants in her experiments was not seriously restricted by the lack of zinc until the flowering stage was reached; the deficiency was then shown by the abscission of leaves and flower buds. The control plants which received zinc grew well, blossomed freely, and set seeds. At maturity, the average dry weight of the bean plants without zinc was 13.3 gm., the weight with zinc 27.0 gm.

In experiments with corn (*Zea mays*) by the present writer and Beck (*9*), it was found that the production of cobs and kernels was relatively more reduced by zinc deficiency than the production of stalks, leaves, and husks. Three consecutive crops of corn were grown to maturity in small tanks of soil, half of which were supplied with nutrients and microelements, except zinc; the other half received the same supplies with the addition of zinc. The effects of zinc were not well defined in the first, but were perfectly evident in the second and third crops. In the third crop the production of cobs and kernels was small.

Bobko and Zerling (*1*) found that the greatest concentration of boron was regularly in the parts of the plant associated with fecundation, and showed that the addition of boron increased the percentage of germination of pollen grains and the length of pollen tubes produced therefrom. Manganese, zinc, iodine, and copper had similar but less obvious effects.

Parker (*8*) applied a zinc sulfate solution prior to the blooming period to grapefruit trees affected with mottle-leaf. He found that, although zinc deficiency had formerly greatly reduced the crop, the application of zinc produced an increase in the quantity and an improvement in the quality of the fruit in the first season after spraying. While there was also an improvement in the vegetative condition of the trees, the improvement in the fruit was so immediate that it could not have been related solely to the development of better foliage. The more severely affected trees in the orchard responded most strikingly to the treatment.

MATERIALS AND METHODS

Experiments on the relation of zinc to seed production were carried out with Dwarf Telephone peas, Davis Dwarf Wax beans, and Dwarf milo. The plants were grown to maturity in nutrient solutions made with extreme care. The composition of the nutrient solutions was that given by Stout and Arnon (*14*), except the concentrations of zinc. As shown in figure 1, one set of cultures received all the microelements except zinc, others received 0.005, 0.02, 0.10, and 0.20 parts per million of zinc. Dwarf milo plants grew so large as to need additional applications of zinc at various intervals. The original amounts added were those stated above, but the applications were repeated three times, giving, therefore, four times the original amount of zinc furnished, which was necessary to keep the plants in a good state of health. All experiments were made in pyrex glass beakers, in a well-lighted greenhouse during the summer months, in Berkeley, Calif.

The amount of zinc in samples of the original seeds was kindly determined by Dr. P. R. Stout, by means of the polarigraph. The peas contained from 8.1 to 8.5 gammas per seed, or an average of 25 p. p. m. of air-dry weight. The milo seeds contained from 0.63 to 0.67 gammas per seed, or, expressed on the basis of air-dry weight, from 20.1 to 21.1 p. p. m. The seeds were washed in redistilled water to remove adhering particles of dust from the surface, and germinated in acid-washed sand. They were later transferred to the special plaster of paris covers utilized to support the seedlings in the beakers. Three plants were usually installed in beakers, each containing 2 liters of the appropriate nutrient solution.

It was necessary to conduct air through the nutrient solution in order to maintain a healthy growth of roots of peas. This was done by blowing a fine stream of air through a sintered glass tube made especially for the purpose. In the nonaerated cultures, the roots of peas became gelatinous and died within about 3 weeks from the time that the experiment was started. The wax beans and milo grew better without aeration than peas, but both responded by better growth when aerated. Since both peas and beans are normally self-pollinated, they served as good test subjects. The dwarf milo, which is supposed to be wind-pollinated, received insufficient pollination to form seeds under the conditions of these experiments and the plants were not as reliable indicators of the effects of zinc as the peas and beans. The weight of the heads of the milo plants was taken, however, since it seemed to be indicative of the processes leading up to seed formation. When each experiment was terminated tops, roots, pods, and seeds were separated and weighed. The seeds that were to be tested for germinating power were dried in the air; the other parts were dried in the oven at temperatures near 100° C.

The value of the several variables are expressed as percentages of the sums of each variable (fig. 1). This method of expression makes it possible to compare the values of the dependent variables on a uniform basis. As a necessary adjunct, the analyses of variance are presented in table 2, without which it would be impossible to estimate the reliability of the results of varying the amounts of zinc furnished the plants.
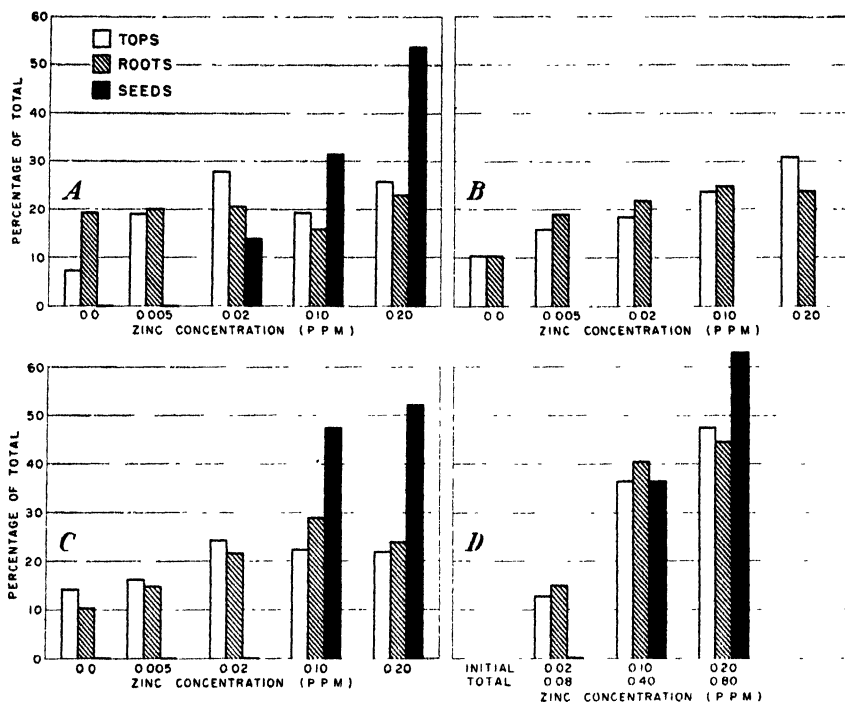
FIGURE 1.—Weight of tops, roots, and seeds of peas, wax beans, and dwarf milo as affected by the quantity of zinc supplied in the nutrient culture: *A*, Dwarf Telephone peas, aerated (experiment 267); *B*, Davis Dwarf Wax beans, aerated and nonaerated combined (experiment 276); *C*, Davis Dwarf Wax beans, aerated (experiment 285); *D*, dwarf milo (experiment 346).

EXPERIMENTAL RESULTS

PEAS

In experiments to determine the effect of zinc on the production of seed, Dwarf Telephone peas were grown in water cultures, some of which were aerated and others unaerated. The cotyledons were removed when the seedlings were transferred to the cultures. In each set of cultures there were 15 beakers containing nutrient solution. The controls contained all microelements except zinc; the other cultures received 0.005, 0.02, 0.10, and 0.20 p. p. m. of zinc.

Seedling peas were installed in the pyrex beakers on May 24 according to the technique outlined above. For a short time they made very little growth, probably because of the removal of the cotyledons, but later they grew better and reached stages of development obviously related to the supply of zinc. Without forced aeration the roots were unable to grow for more than a short time. On June 14 the roots of plants in nonaerated cultures were dead and gelatinous and consequently the tops had made less growth than those of the plants in aerated cultures. All plants, however, were beginning to form flowers on that date. The plants that grew in the aerated cultures were harvested on July 31 and the seeds counted.

The important relations of zinc to the growth of the pea plant (tops and roots) as well as to the seeds are shown in figure 1, A. Increases in the supply of zinc were reflected to a slight degree in the dry weight

of tops, but not at all in the weight of roots when the weight in each culture was expressed as a percent of the combined weight of all cultures. The number of seeds or their weights, on the contrary, show a remarkably close relation to the amounts of zinc supplied (table 1). In the presence of 0.005 p. p. m. of zinc, there was no production of seed, but in 0.02 p. p. m. a significant number of seeds were produced, and at the higher concentrations seed production was materially augmented.

TABLE 1.—*Effect of the quantity of zinc supplied on the average number of seeds produced per culture by Dwarf Telephone peas and Davis Dwarf Wax beans*

Experiment No.[1]	Plants and treatment	Seeds produced when indicated quantity of zinc was supplied (p p. m)				
		0	0 005	0 02	0.10	0 20
		Number	*Number*	*Number*	*Number*	*Number*
267	Dwarf Telephone peas (aerated)	0	0	13	21 3	38 0
276	Davis Dwarf Wax beans (aerated)	0	0	3	34 3	28 0
	Davis Dwarf Wax beans (nonaerated)	0	0	2	44 7	27. 7
285	Davis Dwarf Wax beans (aerated)	0	0	0	17 3	16 0

[1] In experiments 267 and 276 each figure is the average of 3 cultures of 3 plants each, in experiment 285 of 6 cultures of 3 plants each.

The probabilities were more than 99 to 1 that the differences in the weights of seeds and of number of seeds were real differences (table 2); but differences in the dry weights of roots were not statistically significant.

Further insight into the functions of zinc can be obtained from the following observations on the viability and growth of the seeds produced in this set of cultures. A high percentage germinated from all except the minus-zinc cultures (table 3), in which the single seed that germinated produced only a small depauperate plant. Figure 2

FIGURE 2.—Seedlings produced from seeds of pea plants grown in experiment No. 267 in nutrient cultures containing different quantities of zinc: *a*, Beakers 1-3. parent plants received no zinc; *b*, beakers 4-6, parent plants received 0.005 p. p. m of zinc: *c*, beakers 7-9, parent plants received 0.02 p. p. m. of zinc; *d*, beakers 10-12, parent plants received 0.1 p. p. m. of zinc: *e*, beakers 13 15, parent plants received 0.2 p. p. m. of zinc. Plants photographed June 20, 1939, 20 days after planting. See table 3.

shows the vigorous seedlings that came from these seeds despite the fact that some of the series received very small amounts of zinc and the seeds produced were not filled out as well as those in the normal plants. The cotyledons of the parent plants had been, as previously stated, removed at the time the plants were installed in the water cultures.

TABLE 2.—*Analysis of variance between treatments for plants that received different quantities of zinc in their nutrient cultures*

Plant, experiment No., and item compared	Degrees of freedom	Mean square between treatments	F	Value of F required for probabilities of—	
				95:5	99:1 [1]
Peas, experiment 267:					
Dry weight of tops	4	41.79	5.30	3.48	5.99
Dry weight of roots	4	.23	2.96	5 96	
Dry weight of seeds [2]	2	46.70	44.06		10.92
Number of seeds [2]	2	470.11	53.56		10 92
Beans; experiment 276:					
Dry weight of leaves	4	2 96	7.45		4 43
Dry weight of roots	4	.52	4.03	2 87	4.43
Number of pods	4	327.00	18 33		4.43
Number of seeds [3]	1	408.34	3.73	5 32	
Beans; experiment 285:					
Dry weight of leaves	4	9.21	7 61		4 18
Dry weight of roots	4	9 17	19.30		4 18
Dry weight of pods	4	31.60	2,351.56		4 18
Dry weight of seeds [3]	1	.65	4 58	242 00	
Number of pods	4	55.97	2 43	2 76	
Number of seeds [3]	1	5.34	4 22	242 00	
Dwarf milo; experiment 346:					
Dry weight of heads	2	29 51	9.68		8.02
Dry weight of tops without heads	2	2,232.86	17.19		8 02
Dry weight of roots	2	269 57	11.79		8 02
Dwarf milo; experiment 361:					
Weight of tops	4	7,626.38	67 25		4.89
Dry weight of roots	4	595.06	5 60		4.89

[1] Values of F equal to or exceeding these indicate that differences were statistically highly significant.
[2] Data restricted to cultures that received 0.02, 0 10, and 0.20 p. p. m. of zinc
[3] Data restricted to cultures that received 0.10 and 0 20 p. p. m. of zinc. The differences have no statistical significance.

TABLE 3.—*The germination of peas produced by plants grown in nutrient cultures containing different quantities of zinc; experiment 267*

Beakers No.	Concentration of zinc at the start	Seeds planted	Seeds germinating
	P. p. m.	*Number*	*Number*
1-2-3	0	12	1
4-5-6	.005	15	12
7-8-9	.02	18	17
10-11-12	.10	19	16
13-14-15	.20	18	16

The fact that plants to which no zinc was supplied failed to produce viable seeds is, in itself, emphatic evidence that zinc is essential for the reproductive processes culminating in seed production. Although few seeds were produced when extremely minute amounts of zinc were furnished, their power of germination was good. This can be interpreted as evidence that zinc migrated from the vegetative organs of the plant and accumulated in the seeds.

This latter fact may explain why seeds from plants that received less than 1 part per 10 million of zinc did not differ greatly in viability. The presence of zinc in these peas, although in suboptimal amounts,

might nevertheless cause the formation of sufficient stored auxin to insure the development of the embryonic plants. Skoog (*10*) has demonstrated that the production of auxin is intimately connected with the presence of zinc.

BEANS

An experiment (No. 276) on the relation of zinc to the growth and seed production of Davis Dwarf Wax beans was begun on July 13. The nutrient solution and the concentrations of zinc were the same as those used in the preceding experiment with peas. There were thirty 2-liter beakers, each with three bean seedlings. Fifteen of the beakers were forcibly aerated, 15 nonaerated. On August 14 the beakers were emptied and refilled with nutrient solutions having the original composition. On September 5, owing to depletion of nitrates, there was added 5 cc. of $Ca(NO_3)_2$ molar solution to each beaker in the three lower concentrations, and 10 cc. of the same in the two higher concentrations. All plants began to form pods September 5, although the plants in the lowest concentrations were small. The plants in the 0.10-p. p. m. concentration of zinc were larger and produced more seeds than those in other concentrations.

The plants in experiment 276 were harvested September 26 and records were made of the dry weight, number of pods, number of seeds, etc. Several pods were formed in the minus-zinc aerated cultures, but none in the corresponding nonaerated cultures. No seeds were formed in any of the pods of plants in the minus-zinc cultures. In general the Dwarf Wax beans were better adapted for this type of culture experiment than the peas. The combined yields of aerated and nonaerated cultures are shown in figure 1, *B*, and in table 1, and their statistical reliability in table 2.

All plants in solutions having concentrations of 0.02 p. p. m. of zinc produced pods, but, with few exceptions, they were seedless. The plants in solutions having a concentration of 0.10 p. p. m. of zinc attained the best development, except with respect to leaf weight. In numbers of pods and seeds the plants in this concentration were decidedly superior, as shown by the fact that they produced about 35 percent of the total number of pods and 58 percent of the total number of seeds. The weights of leaves and roots were not, however, much greater than on the plants in 0.02 p. p. m. concentration.

There was a special value between 0.02 and 0.10 p. p. m. of zinc with respect to seed production. This indicates that a higher threshold level in concentration of zinc was needed for seed than for pod production. The root, seed, and pod production in 0.20 p. p. m. was not significantly different from that in 0.10 p. p. m.; the weight of leaves was somewhat greater, but the weight of roots was not significantly different.

Another experiment (No. 285) with Davis Dwarf Wax beans was set up August 11; the same concentrations of zinc as in the preceding experiments were used. There were 30 beakers in the experiment, all aerated, with three plants in each. On September 5, and again on October 10, potassium nitrate was added to the jars containing the larger amounts of zinc in which the plants had made greater growth and utilized more of the nutrient salts . On September 29 it was noted that superior growth was made by plants in the solutions containing the larger amounts of zinc, while in the solutions lacking zinc or containing only 0.005 p. p. m. growth was inferior,

as indicated by the small leaves and the dead margins of many of the older leaves. Many large bean pods, with many seeds, were found in the higher zinc concentrations. The plants were harvested on October 31.

In this experiment the plants showed a smaller range in the number of pods formed than in experiment 276. The weight of the pods, however, showed interesting relations to the concentration of zinc in the solutions. A statistical analysis (table 2) showed that the average number of pods in different cultures was not statistically significant, but their weight (a reflection of size) was highly significant. The number of seeds and their weight were strikingly related to the concentration of zinc in the nutrient solution, as shown by table 1 and figure 1, *C*. The existence of a special value for seed production, which was mentioned previously, was still more strikingly shown in the results of this experiment, where it appears that the special value lies between 0.02 and 0.10 p. p. m. of zinc. In this case the values of seed production in concentrations of 0.20 were not significantly different from those for 0.10 p. p. m. of zinc. This was determined not only by inspection of the graphs, but also by statistical analysis (table 2). The increased weight of tops and roots, corresponding to increasing concentrations of zinc, are quite uniform and parallel, showing no such threshold values in relation to the amount of zinc as in the case of seed formation. This corroborates evidence obtained from previous experiments showing that zinc has a very important role in the formation of fruits and seeds.

MILO

A similar experiment (No. 346) with dwarf milo was started April 24 and terminated August 23. After May 17 the culture solutions were artifically aerated. The original concentrations of zinc having been found inadequate for the milo plants, were supplemented by three subsequent additions which supplied a total of 0.02, 0.08, 0.40, and 0.80 p. p. m. Other nutrients were added on three occasions to replace the amounts that had disappeared.

When the plants in experiment 346 were harvested, August 23, it was noted that many in the solutions lacking zinc, as well as in the solutions of lower concentrations, had died sometime before harvest. All of those still alive were stunted and many showed signs of injury. Some of the plants showed the symptom known as "white bud," a common indication of zinc deficiency in maize and milo (*3*). It was only in initial concentrations of 0.1 and 0.2 p. p. m. of zinc that normal plants bearing heads were produced. The phenomenal increase in the weight of heads, as shown by figure 1, *D*, appeared between an initial concentration of 0.02 and 0.20. The dry weight of roots and tops increased as the amount of zinc increased. The probabilities that the differences were due to treatments are better than 99 to 1 (table 2), and indicate that zinc was therefore a very important factor in producing not only the vegetative plants, but also the reproductive heads.

A second experiment (No. 361) with dwarf milo in water cultures was started on June 27 and terminated October 16 (table 4). The zinc concentrations added were, none, 0.005, 0.02, 0.10, and 0.40 p. p. m., the last mentioned being given in four installments of 0.1 p. p. m. each. Additional KNO_3 was given August 2 and 16, and on

September 5 the beakers were emptied and refilled with fresh solutions.   Plants that had received less than 0.1 p. p. m. grew very little and the majority were dead before the termination of the experiment. Since few of the heads contained seeds, their weights were not separately recorded.   The differences in the weights of tops and roots (table 2) are highly significant, and show plainly the importance of zinc for the growth of this plant.

TABLE 4.—*Effect of the quantity of zinc supplied on the dry weight of dwarf milo plants grown in experiment 361*

[Average weight per jar of 3 plants]

Concentration of zinc supplied (p. p. m.)	Weight of		Concentration of zinc supplied (p. p. m.)	Weight of—	
	Tops	Roots		Tops	Roots
	Grams	*Grams*		*Grams*	*Grams*
0	5 9	2 1	0 10	70 8	21 9
0 005	8 7	5 2	0 40	101 4	29 9
0 02	12 3	6 0			

SUMMARY

The necessity of zinc for the complete life cycle of plants is demonstrated by experiments in which highly purified nutrient salts were employed.   Where no zinc was added, growth proceeded to a certain point but not far enough for seeds to develop.

There was a threshold value of zinc for peas and beans, below which the plants produced only small seedless pods.   Above that value the pods were larger and contained seeds.   The seeds produced were viable, although the parent plants received suboptimal supplies of zinc.

Milo showed definite symptoms of zinc deficiency in the lower concentrations supplied.   There was a definite threshold value of zinc for the development of tops and heads.

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REVERSIONS IN MORPHOLOGY OF NITRITE-INDUCED "MUTANTS" OF ASPERGILLI GROWN ON AMINO ACIDS [1]

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INTRODUCTION

Evidence has been presented that variants or mutants of Aspergilli can be produced by the action of nitrous acid and other compounds destructive of amino groups.[2] Later it was reported that lysine or sodium thiosulfate acted to bring about reversion of these artificially produced forms toward the original morphology.[3] The suggestion was made that this type of mutation (injury) and reversion could be due to loss and recovery, respectively, of ε-amino groups in lysine units of the fungus proteins. In view of the considerable amount of evidence relative to interconversion of amino acids and possible transaminations, further tests were considered desirable.

EXPERIMENTAL TECHNIQUE

The nitrite-induced variants of the Aspergilli selected for additional study were the "yellow-woolly" strain (N1), obtained from *Aspergillus niger* Van Tiegh. (No. 4247), and a "woolly nonperithecial" strain, obtained from *A. amstelodami* (Mangin) Thom and Church (No. 126). These have already been described.[2] The former produces masses of mycelium with very few spores and has a yellow reverse. The latter forms an abundance of aerial hyphae covered with many green spores but with perithecia reduced to very few. They were grown in 200-cc. Erlenmeyer pyrex flasks on 50 cc. of base solution of the following composition: Water, 1,000.0 cc.; sucrose, 50.0 gm.; ammonium nitrate, 1.90 gm.; dipotassium phosphate, 0.35 gm.; and magnesium sulfate (7H₂0), 0.25 gm. Iron, zinc, copper, manganese, and molybdenum were added in concentrations of 0.20, 0.20, 0.05, 0.025, and 0.02 mg. per liter, respectively. The amino acids were added to this solution, as required, at a concentration of 10.0 gm. per liter, together with 20 gm. of calcium carbonate (1.0 gm. per flask). The *A. niger* (N1) cultures were kept at 35° C., the *A. amstelodami* (A1) at room temperature. Duration of growth was from 30 to 50 days. During this time transfers were made to Czapek agar slants at varying intervals to determine whether any variants had arisen.

In the experimental production of variants, transfers were made from any portion of the culture showing a contrast in morphology, particularly also from patches of sterile aerial hyphae. In attempts

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to obtain reversions to type, transfers were made with spores of typical appearance whenever possible. Transfers were made first to Czapek agar slants and were continued with material from single heads until the cultures seemed to be pure.

The standards, or types, for estimating morphological variation were the original strains from which the stock variant cultures (N1, A1) had been isolated after being grown on sodium nitrite.

REVERSIONS WITH AMINO ACIDS

The results of studies on the ability of amino acids to cause reversions are summarized in table 1. These studies included trials (duplicate cultures) with the amino acids when used singly or in combination with lysine. A total of 38 variants was isolated after growth of the N1 strain of *Aspergillus niger* on the individual amino acids. Of these variants, 4 were typical *A. niger* strains, and 23 were more nearly typical in appearance than the N1 strain. In admixture with lysine, 44 variants were produced of which 6 resembled the original strain very closely in appearance and 29 resembled it more closely than did the N1 strain. One of the strains isolated from an N1 culture containing lysine together with histidine was found on comparison to be practically identical with the mutant obtained by Schiemann [4] with potassium bichromate and named *A. cinnamomeus* because its spores were brown instead of black.

The corresponding totals for *Aspergillus amstelodami* (A1) without lysine were 18 variants, of which none exactly resembled the standard strain, but 16 had a closer resemblance than was shown by the A1 strain. In combination with lysine, 23 variants were isolated, of which 1 was a typical *A. amstelodami* and 19 were less atypical than the A1 strain. The single typical *A. amstelodami* strain was obtained following growth on a mixture of lysine and threonine. Its isolation partook of the nature of a fortunate chance, since the growth was small and quickly submerged. Its identification was possible only because of the system of daily examinations employed.

The differences between the use of amino acids with and without lysine are definitely in favor of the admixtures in aiding reversions. The figures are not impressive, however, as indicative of a marked variation in effectiveness. Nevertheless these values do not reflect the extent to which reversions seemed to have taken place in the two series in the individual isolated strains. On this basis there could be little question of the superiority of admixture with lysine. Complete reversion to standard forms occurred in the absence of an amino acid supply in one instance when addition of calcium carbonate was omitted. In the presence of calcium carbonate the N1 and A1 strains produced no variants without a supply of amino acid.

The reverted strains that were isolated from cultures of the "yellow-woolly" strain of *Aspergillus niger* varied considerably in appearance though many of the cultures seemed to be quite similar. The reverse of the mycelial felts varied from white to black, to pink, and to chocolate. The quantity of spores and aerial hyphae also varied greatly. The standard or typical strain was black with spores, aerial hyphae were practically absent, and the reverse of the mycelial felts was usually light tan.

[4] SCHIEMANN, ELISABETH. MUTATIONEN REI ASPERGILLUS NIGER VAN TIEGHEM. Ztschr f. Induktive Abstam. Vererbungslehre 8.1 –35, illus. 1912

TABLE 1.—*Variants obtained from cultures of nitrite-induced mutants of Aspergillus niger and A. amstelodami after growth on amino acids*

Amino acid (10 0 gm. per liter)	Variants with *Aspergillus niger* "yellow-woolly" strain (N1)						Variants with *Aspergillus amstelodami* "woolly nonperithecial" strain (A1)					
	Minus *d*-lysine			Plus *d*-lysine			Minus *d*-lysine			Plus *d*-lysine		
	Total	Reversion complete	Reversion incomplete	Total	Reversion complete	Reversion incomplete	Total	Reversion complete	Reversion incomplete	Total	Reversion complete	Reversion incomplete
	No	*No.*	*No*	*No.*	*No.*	*No.*	*No*	*No.*	*No*	*No*	*No.*	*No.*
dl-Alanine	1	0	1	1	0	1	0	0	0	1	0	1
d-Arginine hydrochloride	3	0	2	6	1	4	1	0	1	2	0	2
l-Aspartic acid	2	0	2	2	1	0	0	0	0	1	0	1
l-Cystine	2	1	1			----	1	0	1			----
d-Glutamic acid	2	0	1	1	0	1	1	0	1	1	0	1
Glycine	2	0	1	2	0	1	1	0	1	1	0	1
l-Histidine dihydrochloride	3	0	1	2	1 2	0	2	0	2	2	0	2
l Hydroxyproline	1	0	0	1	0	0	1	0	0	1	0	0
dl-Isoleucine	2	0	2	5	0	4	1	0	1	1	0	1
l-Leucine	0	0	0	2	0	2	2	0	2	1	0	1
d-Lysine dihydrochloride			----	2	0	2			--	1	0	1
dl-Methionine	0	0	0	2	0	2	0	0	0	1	0	1
dl-Norleucine	2	0	1	2	0	0	0	0	0	1	0	1
dl-Phenylalanine	2	0	1	1	0	1	0	0	0	0	0	0
dl-β-Phenyl-*β* alanine	3	1	2	2	0	2	2	0	2	1	0	1
l-Proline	0	0	0	0	0	0	0	0	0	2	0	0
dl-Threonine	4	1	3	3	0	3	1	0	1	2	1	1
l-Tryptophane	3	0	3	4	0	3	1	0	1	1	0	1
l-Tyrosine	1	0	0	2	1	1	1	0	1	0	0	0
dl-Valine	2	1	1	2	1	1	2	0	2	1	0	1
Control [2]	0	0	0				0	0	0			-
β Alanine	3	0	1	1	0	0	1	0	0	1	0	1
Cysteine hydrochloride			--	1	0	1			---	1	0	1
Total	38	4	23	41	6	29	18	0	16	23	1	19

1 of these strains was identified as *Aspergillus cinnamomeus*.
[2] No amino acid.

Changes of a similar nature took place with *Aspergillus amstelodami*. The most marked of these changes were associated with enhancement of perithecial formation. Interesting atypical forms also occurred that produced a broadly spreading, more or less woolly colony, upon which short conidiophores were produced in abundance along trailing hyphae and ropes of hyphae. These conidiophores were short, the heads small; the whole colony became dark green; perithecial development was inhibited or suppressed; and none of the orange color so characteristic of the species was evident.

The great frequency with which apparently identically reverted forms were isolated gave the impression that, while experimental manipulation of environmental conditions influenced these changes, the more important factor was the internal mechanism of the organism. Internal instability might be increased by amino acids and perhaps guided somewhat in the direction of the original morphology, but the changes that occurred were in all directions within the limits of the species. It is quite possible, on the other hand, that continued investigation along these lines may lead to a better understanding of the mechanisms involved and the development of reagents of much greater specificity.

Results obtained in the parallel series of table 1 are complicated by the fact that the strains selected for use did not remain entirely un-

changed. The "yellow-woolly" strain of *Aspergillus niger*, after 23 transfers during 3 years, no longer was yellow on its reverse side and aerial hyphae had become profuse, whereas the *A. amstelodami* strain became completely free from perithecia during this interval. The change with *A. niger* began on the twenty-first transfer. The further adjustments in morphology of these atypical strains, or "injury mutants," were in a direction toward greater abnormality and seemed to be accompanied by a greater resistance to the action of the amino acids.

Each strain was tested once with the following miscellaneous organic compounds. Amino acids were not added. Only with nicotinic acid did reversion to a typical strain occur in *Aspergillus niger*. Changes toward complete reversion (spores) were also exhibited by strains isolated from cultures with pyrazine-2,3-dicarboxylic acid; 2-methyl-1,4-naphthoquinone; calcium *d*-pantothenate; biotin concentrate; β-carotene; ascorbic acid; thiamin chloride; and *i*-inositol. Loss of yellow color had taken place in strains isolated from cultures with pyrazine monocarboxylic acid, calcium pantothenate, biotin concentrate, pyruvic acid, ascorbic acid, and *i*-inositol. No forms of differing characteristics could be isolated from cultures containing phthiocol, α-tocopherol, sodium hyposulfite, lecithin (egg), nicotinic acid amide, sodium-iron chlorophyllin, riboflavin, pimelic acid, and vitamin B_6.

In the *Aspergillus amstelodami* series partially reverted strains were obtained with biotin concentrate, α-tocopherol, pimelic acid, vitamin B_6, and thiamin chloride. All were similar in appearance and characterized by an abundance of perithecia, spores, and aerial hyphae. The original or standard strain was practically free from aerial hyphae.

It seemed possible that through the use of admixtures additional evidence might be obtained as to whether internal instability or determinative influence of amino acids was the predominant factor in producing these results. That is to say, a mixture of amino acids and accessories, in the presence of each of whose constituents a reversion to standard had taken place, should give results much superior to those obtained with the individual compounds. This would not follow invariably, since mutual inhibition might occur but should not take place in every instance. Results with admixtures followed the usual pattern, ranging from recovery of the mutant only to recovery of strains showing complete reversion. The best results were given by a mixture of *d*-lysine, *dl*-valine, and nicotinic acid with the *Aspergillus niger* mutant. Six variants were obtained from a single culture, including (1) reversion complete, smooth white reverse, (2) reversion complete, rough white reverse, (3) reversion complete, black reverse, (4) reversion complete, red to chocolate reverse, (5) white woolly with many spores, and (6) woolly, almost normal sporulation, smooth pink reverse. The substitution of tyrosine for valine led to the isolation of an almost fully reverted strain with red to chocolate reverse. . Calcium carbonate was not added to these cultures. Results with mixtures were negative with *A. amstelodami* (A1), and gave poorer results than usual, since not a single strain showing any degree of reversion appeared.

UTILIZATION OF AMINO ACID NITROGEN

It seemed desirable in conclusion to compare the growth responses of a variant to amino acids with those of the original or standard strain. To do so might throw some light on the cause of the action of amino acids in favoring reversions, and on a possible relation between capacity for amino acid assimilation and normalcy. The "yellow-woolly" strain of *Aspergillus niger* was selected for this purpose because the amino acid nutrition of the standard strain had already been studied.[5] Data on a third strain, "slightly atypical," were also obtained (table 2). This had been isolated from stock cultures of the standard strain after continued propagation for several years on a medium containing 1 percent each of peptone, sucrose, and agar, together with traces of Difco peptone and malt extract. It was characterized by lower yields, greater acidification of the substrate, and higher starch production. In appearance it resembled the standard strain very closely when grown on Czapek agar.

TABLE 2.—*Utilization of nitrogen sources by a standard strain of Aspergillus niger and by 2 of its "mutants" during 4, 5, and 11 days of growth at 35° C.*

Nitrogen source	Standard strain [1]		Slightly atypical strain		"Yellow-woolly mutant" (N1)			
	Yield after 4 days	Sporulation [2]	Yield after 4 days	Sporulation [2]	Yield after 5 days	Sporulation [2]	Yield after 11 days	Sporulation [2]
	Mg.		*Mg.*		*Mg.*		*Mg.*	
dl-Alanine	1,115.8	10	596.7	4	160.0	0	552.0	0
d-Arginine hydrochloride	1,252.4	10	968.0	4	150.9	0	520.4	0
l-Aspartic acid	1,325.1	10	1,130.0	6	209.9	0	611.4	0
l-Cystine	71.0	1	70.5	1	108.5	0	255.3	0
d-Glutamic acid	1,292.8	10	1,196.5	10, bb	187.6	0	524.5	0
Glycine	1,234.8	10	933.8	4	115.0	0	503.6	0
l-Histidine dihydrochloride	120.9	4	213.9	4, t	73.7	0	591.7	0
l-Hydroxyproline	1,147.4	10	747.3	2	5.2	0	44.9	0
l-Iodogorgoic acid	0	0						
dl-Isoleucine	246.2	4	84.8	0	42.8	0	270.5	0
l-Leucine	373.6	6	160.1	0	11.0	0	326.4	0
d-Lysine dihydrochloride	16.2	0	14.7	1	8.1	0	196.6	0
dl-Methionine	437.0	7	273.2	0	0		414.8	0
dl-Norleucine	113.8	1	91.0	0		0	223.6	0
dl-Phenylalanine	370.8	4	206.0	1	2.7	0	280.8	0
l-Proline	1,110.7	10	1,019.0	8	115.2	0	531.9	0
dl-Serine	608.6	7	723.2	0	83.9	0	384.7	0
dl-Threonine	647.4	6	345.5	0	95.1	0	386.3	0
l-Tryptophane	636.3	6	555.9	2			351.4	0
l-Tyrosine	196.1	10	227.9	6	110.0	0	310.9	0
dl-Valine	291.8	1	186.9	0	80.4	0	315.9	0
Cysteine hydrochloride	12.2	0	10.2	0			21.2	0
δ-Alanine	18.7	4	29.5	3, br				
Ammonium nitrate	1,213.2	10	709.8	2	139.7	0	677.5	2

[1] Data from Steinberg (see footnote 5)
[2] Sporulation is rated from 0 (sterile) to 10 (covered with spores) Where spores were other than a normal black color, they are indicated by bb (black and brown), br (brown), and t (tan).

The responses obtained respecting utilization of amino acids would indicate that incapacity for normal development of the variant strains is accompanied by a similar incapacity to employ these acids in a normal manner. Inhibition in the utilization of amino acid nitrogen is relatively greater with aspartic acid, glutamic acid, and proline. These are the fully plastic acids, so-called because best suited to supply both nitrogen and carbon to the standard organism. The

[5] STEINBERG, ROBERT A. EFFECT OF TRACE ELEMENTS ON GROWTH OF ASPERGILLUS NIGER WITH AMINO ACIDS. Jour. Agr. Res. 64. 455–75.

relatively higher yields with the practically unassimilable or aplastic acids (cystine, histidine, lysine, norleucine, and tyrosine) are probably due to some extent to an incapacity to employ the plastic acids. The "yellow-woolly" strain had lost the capacity to utilize hydroxyproline, whereas histidine sufficed to give maximum yields. It had also become a high producer of starch. The immediate causes of these altered responses will probably be found in a disturbance of the basic enzymatic complement of the original organism from which this "injury mutant" was obtained with nitrite. The presence, for example, of a greater content of histidase in the variant as compared to the original strain could lead to these results. The glutamic acid and ammonia formed from histidine would give improved growth. An interesting phase of this question is concerned with the possibility of so causing formation of new enzymes. Such changes perhaps occur but are still to be demonstrated.

On the whole, however, the distinguishing features of the amino acid responses by the standard strain are also recognizable in the aberrant strains. Those acids best assimilated by the standard strain were among those best assimilated by the variant strains. Those most poorly utilized by the standard strain were among those least readily made use of by the variant strains. In general, both variant strains exhibited a decreased capacity to employ the fully plastic amino acids and an increased capacity to use the aplastic acids. These modifications may be the underlying cause for their decreased rate of growth. The degree to which modifications in amino acid responses were obtained corresponded broadly with the extent to which morphological alterations had occurred in the mutants. The deterioration in capacity of the slightly atypical strain to employ inorganic nitrogen as compared to organic nitrogen is indicative perhaps of the possibility of producing a strain of *Aspergillus niger* incapable of assimilating inorganic nitrogen. Such a change has been accomplished with bacteria.

DISCUSSION

Experiments having for their purpose the study of the action of a supply of normal cell constituents can be considered to a great extent as concentration studies. At no time can these compounds be viewed as completely absent from the organism. It is not strange, therefore, that experimental reversions not only are induced through addition of amino acids to the substrate but also occasionally arise spontaneously. Abnormal metabolism of cells, leading to localized accumulations of the individual amino acids, may be a factor in the occurrence of such spontaneous reversions. Tryptophane has been found capable of causing the formation of galls in green plants.[6] Whether amino acids are the primary cause of these variations or are effective only indirectly remains to be determined.

The evidence gathered in trials with the amino acids would appear to indicate that reversions may arise in the presence of other amino acids than lysine. Though the data on which a special role for lysine was assumed is thereby weakened, this amino acid is not entirely eliminated as a possible primary factor. Its presence is conducive to best results, whether its action be direct, as formerly postulated, or

[6]KRAUS, E. J. HISTOLOGICAL REACTION OF BEAN PLANTS TO L-TRYPTOPHANE. Bot. Gaz. 102: 602-622, illus. 1941.

indirect through interconversion or transamination. No data are available at the present time on the ability of amino acids to serve in vitro for replacement of amino groups in deaminized proteins.

Reversions to the original morphology took place with the *Aspergillus niger* N1 strain when grown on lysine, cystine, β-phenyl-β-alanine, threonine, and valine; or in admixture with lysine, on arginine, aspartic acid, histidine, tyrosine, or valine. A mixture of nicotinic acid, lysine, and valine gave still better results. The *A. amstelodami* A1 strain gave reversion to the original morphology only with a mixture of lysine and threonine. It is doubtful, therefore, whether nitrogen nutrition as ordinarily conceived will be found to be the immediate cause of this phenomenon. Excepting arginine, histidine, and aspartic acid, the nine acids most effective in bringing about reversions are least efficient as sources of nitrogen for the N1 strain of *A. niger*.

Intensive studies with a single variant on a much larger scale than was feasible in these investigations should lead to a clearer answer concerning the process of inheritable variations. Larger scale experiments would afford material for statistical evaluation and so help to distinguish between spontaneous and induced changes. Accumulation of additional data with at least every possible combination of amino acids in pairs is advisable. Nor should other cell metabolites be neglected inasmuch as the available facts would indicate that the primary cause of these alterations is injury to the mechanism of cell metabolism. However, no further studies in this field are contemplated by the writers, since conditions make necessary their discontinuance.

Information on the loci of injury is so slight that only speculation is possible on this phase at this time. The data indicate that variations in enzymatic content have taken place, and perhaps some formed de novo. A study of the shifts observed in the normal pattern of amino acid utilization accompanying inheritable structural variations due to injury should prove a valuable tool in morphological investigations. They point to a direct interrelation between the enzymatic complement of the organism and morphological normalcy. This correlation is not unexpected, since inheritance must have its fundamental basis in nutrition, and this study and those of earlier workers would substantiate this interpretation. Studies of this type, therefore, provide a means for obtaining direct information concerning the relation of metabolic capacities to structure and development and of their bearing on inheritable variations. Cell maturation in animals is also interfered with by carcinogenic compounds.

SUMMARY

Reversions to the morphology of the original strain took place with a nitrite-induced injury-mutant of *Aspergillus niger* Van Tiegh. when grown on lysine, cystine, β-phenyl-β-alanine, threonine, and valine; or in admixture with lysine, on arginine, aspartic acid, histidine, tyrosine, or valine. A mixture of nicotinic acid, lysine, and valine gave the best results. A nitrite-induced injury-variant of *A. amstelodami* (Mangin) Thom and Church gave complete reversion to the morphology of the original strain only with a mixture of lysine

and threonine. Assimilability of amino acids did not appear to be a major factor in these responses.

Comparison of capacities for amino acid utilization between the standard strain and each of two variant strains of *Aspergillus niger* disclosed alterations proportional to the extent of morphological change. These changed assimilation capacities in the variants did not destroy the general nature of the responses characteristic of the original strain, though individual amino acids were affected. The variant having greater alterations in morphology showed much less capacity for assimilation of the fully plastic acids (aspartic acid, glutamic acid, and proline) and increased capacity for utilization of the aplastic acids (cystine, histidine, lysine, norleucine, and tyrosine). Utilization of hydroxyproline had become particularly poor in the almost sterile or more atypical variant. Enzyme suppression with nitrite disturbed cell maturation as in abnormal cells produced in animals by carcinogenic compounds.

MICROBIOLOGICAL AND NUTRITIONAL FACTORS IN THE TAKE-ALL DISEASE OF WHEAT[1]

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INTRODUCTION

Numerous soil amendments (*3, 5, 8*)[3] as well as tillage practices (*13*) have been reported of value in controlling the take-all disease of wheat (*Triticum aestivum* L.), caused by *Ophiobolus graminis* Sacc. In many instances procedures effective in one locality are ineffective in another, or even in the same region or field in different years. The spontaneous disappearance of diseased areas in fields has been noted (*4*). The extent to which manurial and tillage practices are effective and the precise mechanism of their influence on the incidence of the disease remain controversial. Organic amendments have been generally successful in providing control even under greenhouse conditions, where some treatments effective in the field fail. It has been suggested (*3, 5, 11, 14*) that organic amendments reduce the severity of take-all disease because they stimulate antagonistic or competitive micro-organisms in soil.

Previous work at this laboratory (*1, 2*) has shown that organic amendments applied to soil have no apparent effect on root-surface microfloras, even though markedly stimulating the soil microflora. It was suggested that, once the root parasite reaches the susceptible plant, factors of nutrition, host resistance, or parasite virulence are probably more important than microbial antagonisms. But nutritional aspects of organic manures have been considered of little importance. It has been stated that the effects of animal manures are due to their action on the parasite and not to their benefits to the host plant (*10*). Soils of the eastern-central region of Kansas in which take-all disease occurs are frequently low in available phosphorus (*16*), and phosphatic fertilizations have at times been sufficient to control take-all in the field (*8, 13*).

Garrett (*7*) has suggested that the presence of a suitable nitrogen supply plays an important role in permitting the survival of *Ophiobolus graminis* in soil, and in recent work he considered competition among soil micro-organisms for nitrogen more significant than antagonism. He stated (*6*) as characteristic of organic manures that control take-all that they contain insufficient or just sufficient nitrogen for their de-

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composition. This suggests that the amount of available nitrogen as well as of available phosphorus may be of importance in the incidence or disappearance of *Ophiobolus*. The present investigations were designed to evaluate the importance of microbial activity and of available-phosphorus and nitrate-nitrogen levels of soil as determining factors in the incidence or severity of take-all disease.

METHODS AND MATERIALS

Both field areas and potted experimental soils were employed for study; the greenhouse experiments were established in order to provide more pronounced variations in the factors under study than might be readily encountered under field conditions. Field areas that had histories of take-all disease for several years, and adjacent areas in which take-all damage had not been observed, were selected for study. Soil samples for analyses were collected at intervals throughout several crop seasons and consisted of well-mixed aliquots, as nearly free of roots as possible, taken from the upper 4 to 6 inches of soil at several sampling stations. From certain naturally infested field areas, soil was collected also in larger quantities for greenhouse experiments; for this purpose no attempt was made to avoid the inclusion of diseased root fragments. Each soil lot collected for potting was sieved and thoroughly mixed to insure uniformity in the subsequent aliquot portions required.

The methods employed for determination of numbers and types of micro-organisms have been reported previously (*1, 2*). The method of Truog and Meyer (*17*) was used in the determination of available phosphorus, and the phenoldisulfonic acid method, as modified by Harper (*9*), in the determination of nitrate nitrogen. The pH values, as determined with a glass electrode, of all soils employed fell between 5 and 7. All colorimetric readings were made with a Duboscq colorimeter.

FIELD OBSERVATIONS

For take-all infested and noninfested field areas of the silt loam and clay loam prairie soils studied, differences either in numbers or kinds of micro-organisms were not apparent. The plate-count estimates of either total micropopulations, or of component subgroups thereof, as actinomycetes, filamentous fungi, total bacteria, or dye-tolerant, fluorescent, and spore-forming types of bacteria, showed close agreement on any given date of sampling. To exemplify, bacterial numbers averaged 28.7 million per gram of air-dry soil in eight infested areas and 25.1 million per gram in eight noninfested areas sampled June 14, 1938. Complete seasonal studies showed that at no time was it possible to identify infested areas on the basis of microbial numbers found therein. The isolation and examination of single cultures showed the same bacterial types and species predominant in infested as in noninfested soil; striking differences, such as are evident between soil and root-surface microfloras, could not be observed. Those microbial species commonly recorded as antagonistic in plate culture or pot experiments, as *Bacillus vulgatus*, *Trichoderma* spp., and *Actinomyces* spp., could be isolated with equal facility from either soil condition.

Available-phosphorus levels of take-all infested and noninfested field areas, as revealed by soil samples collected in May and June

1940, are shown in table 1. Although no correlation between the level of available phosphorus and the presence or absence of disease was apparent, it was noted that the available-phosphorus level was 18.3 parts per million of phosphorus in one field for which there was a history of reduction in the severity of take-all disease following phosphatic fertilization.

TABLE 1. — *Available-phosphorus levels in soil of take-all areas and in immediately adjacent field portions or fields on which healthy wheat was grown*

Sample pair No.	Available phosphorus in —		Sample pair No.	Available phosphorus in—	
	Infested area	Noninfested area		Infested area	Noninfested area
	P. p. m	*P. p. m*		*P. p. m.*	*P. p. m.*
1	67 7	69 8	17	31.4	37.2
2	14 3	13 3	18	14 8	17.6
3	19 2	21.2	19	23 7	21.0
4	75.6	51 5	20	44 3	61.4
5	23.2	26 9	21	38 5	58 1
6	14 1	12 7	22	25 6	36.1
7	18 3	18 3	23	103.6	30 8
8	14 0	18.8	24	21 2	23 2
9	25 8	9 0	25	16.4	21 0
10	23 6	20 5	26	20 0	19 0
11	22 4	28.8	27	38 9	28 3
12	41.3	29 2	28	24 2	14 0
13	130 2		29	44 1	47 7
14	41 2	20 4	30	26 0	33 8
15	53 8	49 7	31	12 3	12 5
16	38 0	30 5			

EXPERIMENTAL WORK

EXPERIMENT 1

Naturally infested silt loam was divided into 18 aliquot portions, and each portion was subsequently treated as outlined in table 2. A total of 54 pots, 3 from each soil portion, was established by potting 1,500-gm. amounts in unglazed 6-inch clay pots. Soil was brought to optimum moisture content October 2, 1939, and water was added daily in amounts sufficient to keep the soils near such content until the termination of the experiment. Kanred wheat was planted October 6, and 3 plants were retained in all except 3 pots (soil aliquot 18), which were fallowed throughout the experiment.

Soil samples were removed from each of the 54 pots on October 6 and 27, November 28, January 12, and March 13, of the 1939–40 season. Replicate samples taken October 27 and November 28 were handled separately for microbiological analyses; those of the other dates were composited thoroughly and then treated as single samples. Data concerning the microbial populations on October 27 and November 28 were sufficient to permit analysis for differences in microbial numbers due to soil treatment. A summary of the microbiological data, together with initial soil treatments, is given in table 2.

For chemical analyses, the replicate samples collected on each of the five dates were composited. Laboratory analyses of these composites revealed that maximum differences from the check soil portion were obtained, as might be expected, with maximum applications of the amendments employed. The completion of the experiment revealed (1) that those soil-nutrient conditions usually considered

TABLE 2.—*Influence of soil treatments employed in experiment 1 upon the soil microbial populations of naturally infested silt loam as revealed by samplings, after growing Kanred wheat, 1939*

Soil aliquot No.	Rate of treatment with —			Micropopulations determined per gram of air-dry soil									
	Super-phosphate	Potas-sium nitrate	Ground wheat straw	Total bacteria		Dye-tolerant bacteria		Spores of aerobic bacteria		Actinomycetes		Filamentous fungi	
	P. p. m.	*P. p. m.*	*P. p. m.*	October 27	Novem-ber 28	October 27	Novem-ber 28	October 27	Novem-ber 28	October 27	Novem-ber 28	October 27	Novem-ber 28
				Millions	*Millions*	*Millions*	*Millions*	*Millions*	*Millions*	*Millions*	*Millions*	*Millions*	*Millions*
1	25			64.7	28.8	28.0	4.6	8.1	2.5	7.3	6.7	0.26	0.16
2	50			77.3	33.9	35.7	6.4	9.7	3.7	19.3	7.1	.38	.13
3	100			71.9	38.6	24.3	9.5	*13.5	**4.9	9.5	4.9	.17	.14
4		40		88.8	38.8	25.8	7.5	8.0	2.8	17.4	8.0	.24	.15
5		80		90.2	39.9	34.1	**11.0	7.5	2.9	*21.6	9.4	.35	.24
6		160	1,000	**165.6	**55.6	**85.8	*10.4	8.7	3.6	**24.8	*10.9	**.67	*.47
7		40	2,500	**189.2	**76.4	**120.5	**27.1	*13.2	**5.8	14.5	**12.3	**.93	**.68
8			5,000	62.6	37.5	18.8	6.1	6.5	3.0	15.5	7.7	.25	.19
9				72.9	39.0	24.0	6.2	6.2	3.7	*20.3	9.4	.24	.19
10				65.4	29.6	30.8	6.7	8.4	*4.0	12.1	6.5	.27	.15
11				68.1	30.4	10.8	6.2	7.1	3.6	15.8	8.3	.30	.27
12				107.9	*44.4	25.1	**12.8	8.9	3.5	8.0	8.7	.33	.19
13			1,000	94.6	42.7	44.5	8.3	11.3	3.7	*20.4	**11.6	.49	*.34
14	25		2,500	91.1	*57.4	**63.8	**15.2	8.1	3.3	*22.3	**12.1	*.56	**.44
15	50		1,000	93.4	43.8	43.2	*10.6	9.7	3.8	*23.3	8.5	.35	.23
16	25	40	1,000	90.4	**50.1	38.5	*10.2	8.3	3.0	**27.5	**11.3	.26	.27
17	25	80	2,500	**165.0	*55.7	**66.9	**12.6	7.6	3.0	*27.9	**11.5	*.56	**.46
18		40		91.6	30.6	33.1	3.9	7.4	3.2	*22.9	8.9	.22	.22
Minimum corresponding value:													
Significant				113.6	44.2	47.7	10.0	12.9	4.0	19.7	10.9	.56	.31
Highly significant				130.3	49.5	54.5	11.8	14.6	4.5	23.9	12.4	.66	.36

*Value exceeds the minimum corresponding value, which is significant. **Value exceeds the minimum corresponding value, which is highly significant.

satisfactory for plant growth were not maintained until the wheat matured, even with the heaviest rates of fertilizer applications employed, and (2) that all pots contained badly diseased wheat at maturity. Accordingly, only the chemical analyses of the check soil and the portions receiving the heaviest fertilizer applications need be considered.

The highest available-phosphorus values (27.5, 41.9, 29.6, and 25.5 p. p. m. of phosphorus) for the check and for the heaviest superphosphate, potassium nitrate, and wheat-straw fertilizations, respectively, were observed in the initial sampling of the experiment, and the lowest values (20.5, 29.7, 20.0, and 18.9 p. p. m. of phosphorus) were obtained in the final sampling on March 13. Although the soil that received the heaviest superphosphate fertilization maintained the highest available-phosphorus level throughout, even this level fell to a rather low value during the latter part of the season. The highest nitrate-nitrogen values observed during the fall for the four soil portions named were 13.6, 14.9, 40.2, and 8.0 p. p. m. of nitrogen, respectively, and the lowest, obtained March 13, were 2.0, 3.8, 5.0, and 0.6 p. p. m. Though distinct differences in the nitrate content of the soils existed early in the fall, during the latter half of the experiment all plants showed the yellowing characteristic of nitrogen starvation. The generally low values revealed by the March 13 samples also indicated that plant growth was limited because of low levels of available nitrogen.

During the first week of April 1940, all wheat plants were removed from pots, and the root systems were washed clean. The roots, crowns, and culms of all plants were severely diseased. But since for a considerable period the phosphorus and nitrate-nitrogen content could not be considered as satisfactory, no assumption could be made that plant-nutrient factors were relatively unimportant, even though initial fertilizer applications had been made at rates superficially comparable to those employed in the field. A second experiment was clearly indicated in which different levels of available phosphorus and nitrogen would not only be provided initially but would also be maintained throughout the growing season.

EXPERIMENT 2

Naturally infested silt loam was divided into 12 aliquot portions for treatment. Superphosphate (45 percent P_2O_5) was added at rates of 50, 100, 200, 600, and 1,000 p. p. m. to soil portions receiving no nitrogen treatment, and at these same rates to soil portions receiving, by the end of the experiment, a total of 976 p. p. m. of nitrogen. For determining this latter rate, the approximate amount of nitrogen required for plant growth was calculated, and inorganic nitrogen (two-thirds as potassium nitrate and one-third as ammonium nitrate) was added to provide nitrogen slightly in excess of the calculated figure. The initial application consisted of 40 p. p. m. of nitrogen, and subsequent additions averaging 16 p. p. m. were made at intervals during the growing season, so that by March 11 a total of 350 mg. of nitrogen per wheat plant had been added (table 3).

Initial soil treatments were made September 19, 1940, and two unglazed pots were filled from each soil portion. All containers were then brought to optimum moisture content. Kanred wheat was

seeded September 23, and three plants were retained in each pot. All soils were sampled September 23 and November 13, and the individual samples were analyzed microbiologically. No statistically significant differences were found in saprophytic micropopulations following the inorganic treatments employed (table 4). Further microbiological analyses were considered unnecessary. Complete samples for chemical analyses were taken September 23 and November 13, 1940, and March 20, 1941, and occasional random samples between November 13 and March 20, to check on nitrogen levels only. Nitrate-nitrogen and available-phosphorus levels for the first and last dates of sampling are given in table 3.

TABLE 3.—*Nitrogen and phosphorus applied in fertilizer, plant growth obtained, and severity of take-all damage to Kanred wheat grown in naturally infested silt loam in experiment 2*

Soil aliquot No.	Superphosphate (45 percent P_2O_5) applied	Inorganic nitrogen applied per plant	Oven-dried plant material per pot		Observed severity of take-all damage
			Top growth	Root system	
	P. p. m	*Milligrams*	*Grams*	*Grams*	
19	50		1.93	0.65	Severe.
20	100		2 08	53	Do.
21	200		1 95	53	Do
22	600		2 30	50	Do
23	1,000		2.30	.75	Do.
24			2 10	.90	Do
25		330	11 10	90	Do
26	50	350	14 90	2 63	Moderate
27	100	350	18 00	2.98	Do.
28	200	350	21 35	4.85	Slight.
29	600	350	34.15	6 33	Very slight
30	1,000	⟍350	35 30	7.05	None.

On March 20, 1941, all plants were removed, and the root systems were washed free of soil, care being taken to keep as many roots as possible attached to the plants. Clean roots and plants were examined for disease lesions, and oven-dried plant material was weighed. Summaries of nitrogen and phosphorus added, weights of plant materials (tops and roots separately), and comparative severity of take-all damage are given in table 3.

Weights of root material recovered by the procedure given and the severity of plant damage by disease showed a close relationship. Roots of all plants were severely diseased (fig. 1), whether grown in untreated soil, in soil that had received either low or high rates of superphosphate without nitrogen, or in soil that had received nitrogen treatment without superphosphate. The total weight of attached root material recovered from such soil treatments (soil aliquots 19 to 25, inclusive, table 3) was less than 1 gm. per pot, as compared with 7 gm. where control was obtained (soil aliquot 30). An adequate supply of either available phosphorus or nitrate nitrogen alone appeared incapable of effecting an appreciable reduction in the severity of the disease.

The weights of root systems recovered from naturally infested soil supplied with sufficient nitrogen showed direct correlation with the supply of available phosphorus. The beneficial effect of increasingly higher levels of available phosphorus in reducing disease became apparent when nitrogen supply was not a limiting factor (table 3;

TABLE 4.—*Influence of different levels of available phosphorus and nitrogen (see table 3) employed in experiment 2 upon the soil microbial populations of naturally infested silt loam and the soil content of available phosphorus and nitrate nitrogen after growing Kanred wheat, 1940–41*

Soil aliquot No.	Micropopulations determined [1] per gram of air-dry soil									Available phosphorus		Nitrate nitrogen	
	Total bacteria		Dye-tolerant bacteria		Spores of aerobic bacteria		Actinomycetes		Filamentous fungi				
	Sept. 23, 1940	Nov. 13, 1940	Sept. 23, 1940	Nov. 13, 1940	Sept. 23, 1940	Nov. 13, 1940	Sept. 23, 1940	Nov. 13, 1940	Nov. 13, 1940	Sept. 23, 1940	March 20, 1941	Sept. 23, 1940	March 20, 1941
	Millions	*Millions*	*Millions*	*Millions*	*Millions*	*Millions*	*Millions*	*Millions*	*Millions*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
19	53.0	88.2	26.9	15.9	3.8	4.0	8.4	7.6	0.27	33.1	22.5	14.4	0
20	52.8	73.4	27.0	12.5	3.3	4.1	5.0	8.0	.20	43.9	26.7	19.3	0
21	52.5	72.3	35.3	13.7	3.6	4.2	6.2	12.1	.18	58.6	41.7	14.4	0
22	47.5	76.0	18.1	12.1	3.4	4.3	6.2	8.2	.16	125.2	79.5	17.8	0
23	34.3	101.0	16.9	15.4	3.3	3.7	8.1	15.0	.24	196.8	85.3	20.3	0
24	50.0	65.3	13.5	10.1	2.7	4.8	8.3	7.6	.23	24.8	17.0	9.3	16.3
25	38.4	71.4	11.2	8.1	2.2	2.4	7.6	9.6	.32	23.6	16.9	5.0	23.3
26	54.0	83.1	13.3	9.4	3.3	3.5	9.0	8.4	.23	33.6	18.4	33.8	12.3
27	55.0	81.3	12.5	12.4	2.4	3.1	5.9	8.0	.26	42.5	22.9	35.6	2.8
28	43.7	107.0	14.8	13.3	2.6	5.4	7.0	8.5	.25	57.0	53.5	19.8	3.2
29	50.6	95.7	23.1	19.1	2.4	4.2	9.5	8.9	.34	132.2	74.4	34.0	.4
30	56.3	104.4	8.9	16.5					.27	197.4		37.2	

[1] Statistical analyses of micropopulations recorded revealed no significant differences
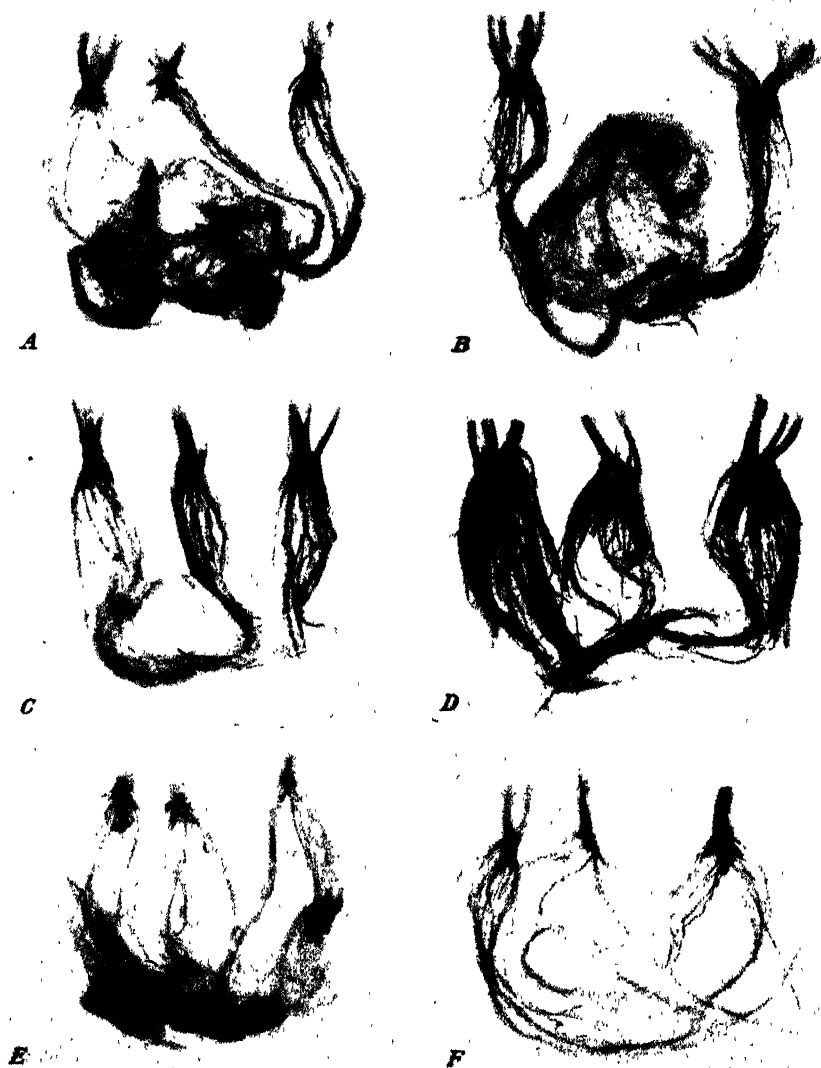
FIGURE 1. --Take-all-diseased wheat roots from untreated soil and from soil that
   received either phosphorus or nitrogen singly: *A* and *B*, Root systems from
   untreated soil; *C*, root systems from soil given 1,000 p. p. m. of treble super-
   phosphate; *D*, root systems from soil given a total of 976 p. p. m. of nitrogen;
   E and *F*, root systems from soil given 50 p. p. m. of treble superphosphate.
   ×0.4.

fig. 2). That excessively high available-phosphorus and nitrate-
nitrogen levels were not required for successful control is indicated by
the terminal nitrogen and phosphorus values shown in table 4. (See
also field levels of available phosphorus given in table 1.) The com-
bination of phosphorus and nitrogen treatments used on soil aliquot 30
permitted development of root systems equal to or superior to those

FIGURE 2.—Wheat roots from soil that received both phosphorus and nitrogen amendments: *A* and *B*, Root systems from soil receiving 50 p. p. m. of treble superphosphate and 976 p. p. m. of nitrogen; *C* and *D*, root systems from soil receiving 1,000 p. p. m. of treble superphosphate and 976 p. p. m. of nitrogen. ×0.36.

developed in aliquots of the same infested soil lot fertilized heavily with chopped green alfalfa (fig. 3), a treatment that has been reported to give good control of take-all disease.

EXPERIMENT 3

Each of eight glazed jars received 3,000 gm. of soil from the same lot as used in experiment 2. The following treatments were employed: (1) Check; (2) 200 p. p. m. of phosphorus applied as superphosphate at planting time; (3) 224 mg. of nitrogen per plant, added to the soil as ammonium nitrate in 60 applications of 4 p. p. m. of nitrogen each

during the growing season; and (4) a combination of phosphorus and nitrogen treatments as cited above. All soils were cropped with three plants each. Wheat, vernalized 30 days at 2° C., was grown for 110 days after vernalization; sufficient artificial lighting was employed to provide a daily plant-illumination period in the greenhouse of 16 hours. Upon removal, all plants grown in the untreated or check soil were found to be severely diseased with *Ophiobolus*, as were those grown in soil receiving phosphorus but not nitrogen. With nitrogen fertilization alone, moderate take-all damage was apparent; with both phosphorus and nitrogen supplied, no take-all damage was evident (fig. 4). The results of experiment 2 were considered confirmed.

FIGURE 3.—Wheat roots from soil amended heavily with chopped green alfalfa. ×0.36.

DISCUSSION

In the early literature on *Ophiobolus*, unsatisfactory soil-nutrient conditions were frequently considered as predisposing causes of take-all disease *(12)*. However, when it was found that inorganic fertilizations failed to give control, especially under greenhouse conditions, host nutrition was assumed to be relatively unimportant, and it was postulated that microbiological antagonism was responsible for the successful control obtained with organic amendments. Although the evidence appears unmistakable that the biological equilibrium of soil does affect the persistence or destructiveness of soil-borne plant pathogens *(6)*, it is equally probable that there are other factors involved which at times may limit the incidence or severity of root rot diseases. The present investigations have indicated that for wheat-cropped, *Ophiobolus*-infested soil, nutritional factors are extremely

important in determining the severity of take-all disease. It is believed that the present work is of special value in permitting clarification of some of the apparently contradictory observations concerning take-all disease.

It has been noted above that phosphatic fertilization frequently but not invariably gives field control of take-all, while similar fertilization under greenhouse conditions has generally been found of no value. The available-phosphorus levels reported for eastern-central Kansas soils suggest that there are some fields in which no reduction of take-all could be expected following phosphorus amendments. In such fields, nitrogen is possibly the limiting factor which predisposes the
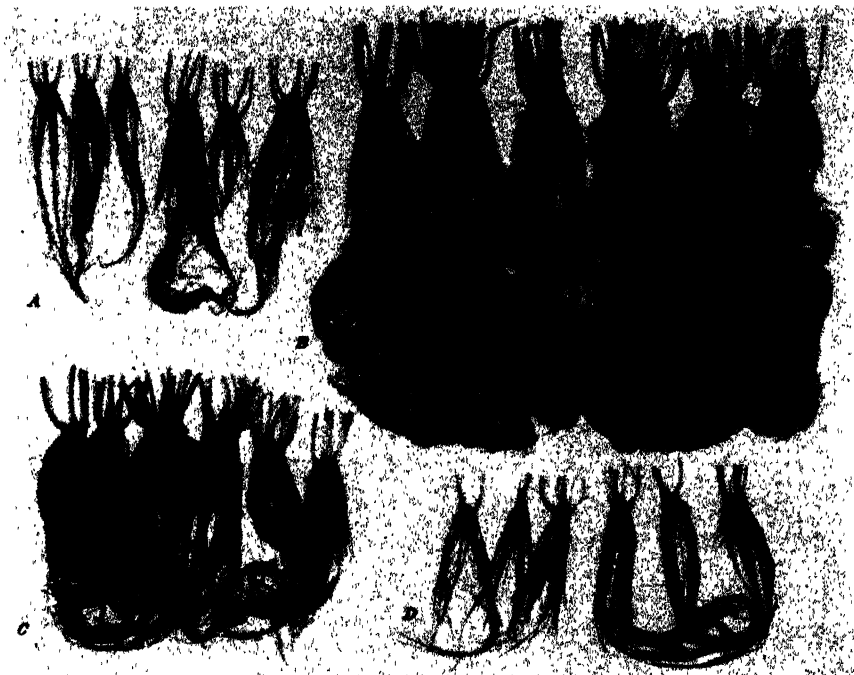
FIGURE 4.— Root systems of wheat plants from experiment 3: *A*, Superphosphate fertilization only; *B*, superphosphate and ammonium nitrate fertilization; *C*, ammonium nitrate fertilization only, *D*, untreated check. ×0.25.

plant to this disease. The frequent failure of phosphatic fertilizations in greenhouse experiments has been shown by the current work to be due to inadequacy of available nitrogen. When the nitrogen content of the soil is maintained at a suitable level, the severity of take-all disease becomes reduced as the available-phosphorus level is raised.

Tillage practices frequently found effective for take-all control may be in part explained by their generally favorable effect upon the accumulation of nitrate nitrogen. That such practices in certain instances fail to provide control may be due to the operation of meteorological or other factors inimical to nitrate-nitrogen accumulation, or to a deficiency of available phosphorus in the soil. Sewell and Call (*15*) have reported upon tillage practices, apparently favorable to accumulation of nitrate nitrogen, which when em-

ployed continuously for several years increased both wheat yield and the severity of take-all disease. It is possible that with the repeated harvesting of heavier yields, the phosphorus deficiency in a soil naturally low in phosphorus might be accentuated to such an extent that the severity of take-all disease would be increased.

The failures reported for inorganic fertilizations under greenhouse conditions are not difficult to understand if one examines both the soil volumes and the rates of fertilizer applications employed. Frequently, only 500 gm. of soil, and occasionally only 200 to 300 gm., per plant have been used (e. g., from 5 to 7 or even 10 plants are reported as grown in unglazed 6-inch pots). This provides only a fraction of the soil volume available to each single wheat plant in the field under normal or optimum stand. That ordinary rates of fertilizer application may not suffice is exemplified by results with potassium nitrate. In field work, 600 pounds per 2,000,000-pound acre is usually considered a heavy application. This same rate (300 p. p. m.) applied to 500 gm. of soil in the greenhouse provides about 18 mg. of nitrogen, or roughly 6 percent of the calculated amount required to produce an average mature wheat plant under field conditions. If all the nitrogen applied at this 300 p.p.m. rate under the greenhouse conditions specified were utilized in plant growth, the response probably could not be detected even if glazed jars were employed. With unglazed pots, losses could be expected because of diffusion of available nitrogen into and from the walls and the bottom openings of such containers. Failure to consider such factors possibly accounts for the fact that control of take-all by means of inorganic fertilizations has not heretofore been reported for greenhouse experiments.

SUMMARY

For wheat-cropped soils of similar texture, differences were not detected in numbers or kinds of micro-organisms in take-all-infested and noninfested field areas, nor was correlation between levels of available phosphorus and incidence of disease apparent.

In greenhouse experiments in which naturally infested soil was given ground wheat straw and inorganic fertilizations at light to heavy field rates at time of planting, the subsequent observations revealed (1) significantly greater soil micropopulations for several months following straw fertilization, (2) unsatisfactory available-phosphorus and nitrate-nitrogen content in soil before the end of the growing season, and (3) at maturity all plants badly damaged by *Ophiobolus graminis*.

When superphosphate fertilization was made at seeding time and inorganic nitrogen, slightly in excess of the amount of nitrogen calculated as necessary for plant growth, was applied during the growing season, it was found that the severity of take-all disease was reduced by increasing rates of superphosphate fertilization. With both available-phosphorus and nitrate-nitrogen content maintained at suitable levels, successful control of take-all disease was obtained, even though significant differences in microbial numbers were not encountered.

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FUNGICIDAL PROPERTIES OF 2,4-DIAMINODIPHENYL-AMINE AND OTHER SUBSTITUTED DIPHENYLAMINES [1]

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INTRODUCTION

Freshly prepared 2,4-diaminodiphenylamine is a colorless crystalline compound that turns brown upon exposure to air. It is slightly soluble in cold water and gasoline and soluble in alcohol, chloroform, benzene, and morpholine. It has a melting point of 123° C., a flash point of 216° C., and a specific gravity of 1.29. In water at 20° C. it is soluble to the extent of 278 p. p. m. It is prepared by reducing 2,4-dinitrodiphenylamine, which is obtained by reacting 2,4-dinitrochlorobenzene with aniline. It has the following structural formula:
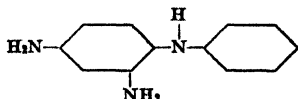
It is used as an antioxidant, an antiplasticizer of unvulcanized rubber, an accelerator of vulcanization, and an activator of other rubber accelerators. It is also used to replace litharge in rubber stocks for insulating wire.

The material is a weak base and is compatible with bentonite-lime mixture, lime, lead arsenate, and calcium arsenate. It is not compatible with nicotine sulfate or rotenone and is inactivated when combined with bentonite and nicotine sulfate-bentonite preparations. It is not easily wetted by water but is easily suspended in water with the help of solvents or wetting agents. It is compatible with sprays containing mineral oils. It is easily handled, without apparent injury to the operator, causes no clogging of spray machinery, and is readily applied when used alone or with adjuvants. Being a dye, it stains the operator's skin and clothes.

In preliminary tests 2,4-diaminodiphenylamine was found to be toxic [2] to several plant pathogens. This paper presents the results of tests to determine the fungicidal properties of this material as well as those of other readily available substituted diphenylamines.

METHODS AND MATERIALS

Since the methods used in the fungicide tests are described fully in previous papers,[3][4] they need be outlined only briefly here. Conidia

of the peach brown rot organism (*Sclerotinia fructicola* (Wint.) Rehm) were obtained from 7-day-old pure cultures growing at 24° C. on sterilized string beans and conidia of the apple bitter rot organism (*Glomerella cingulata* (Ston.) Spauld. and Von Schrenk) were obtained from 7-day-old pure cultures grown at 24° on oatmeal agar.

The conidia were placed in cubes of water agar and perfused with saturated aqueous solutions of the given chemical for 24 hours. During and after perfusion, sample agar cubes were taken out, the conidia that had germinated were counted, and the degree of toxicity of the solution was recorded. Also, glass cover slips bearing standardized spray residues of the chemical, with or without adjuvants, were suspended in special holders from apple trees so that the residues were subjected to weathering under orchard conditions. At frequent intervals these cover slips were brought into the laboratory and the toxicity of the weathered residues was determined by placing conidial suspensions of the test fungi in contact with the weathered residues inverted over Van Tieghem cells and recording the fungicidal effect on germination.

In addition, limited field tests were conducted by spraying apples with 2,4-diaminodiphenylamine at the rate of 2 pounds to 100 gallons of water, with adjuvants, to control scab, caused by *Venturia inaequalis* (Cke.) Wint., and russet; cherries, to control leaf spot, caused by *Coccomyces hiemalis* Hig.; and peaches, to control brown rot, caused by *Sclerotinia fructicola*, and scab, caused by *Cladosporium carpophilum* Thuem. Also, Red Kidney bean plants (*Phaseolus vulgaris* L.) and small limbs of pear, plum, apricot, and quince were sprayed to determine their tolerance.

As only small quantities of the other diphenylamines were available they were subjected only to laboratory tests and to tests for toxicity on the bean plants.

EXPERIMENTAL RESULTS

FUNGICIDAL ACTIVITY OF 2,4-DIAMINODIPHENYLAMINE

PERFUSION AND SPRAY-RESIDUE TESTS

Laboratory studies of the fungicidal properties of saturated solutions of 2,4-diaminodiphenylamine are shown in table 1. The saturated solution, prepared by shaking 2 gm. in 2,000 ml. of distilled water, was brown in color, had a strong but not objectionable odor, and contained approximately 278 p. p. m. of the chemical. Its toxicity (table 1) was compared with that of a saturated solution of diphenylamine, which contained 11 p. p. m., and with that of a solution of copper sulfate, which contained 0.50 p. p. m. of Cu, a concentration often present in water in contact with residues of alkaline bordeaux mixtures.

The saturated solution of 2,4-diaminodiphenylamine was extremely toxic to the conidia of *Sclerotinia fructicola* and *Glomerella cingulata* under these conditions, and that of diphenylamine was more toxic than that of copper sulfate.

Table 2 contains data on the toxicity of residues of 2,4-diaminodiphenylamine, combined with various adjuvants, to the conidia of *Sclerotinia fructicola* and *Glomerella cingulata*, as compared with the toxicity of spray residues of bordeaux mixture under the same con-

ditions of orchard weathering. It also shows that 2,4-diamino-diphenylamine residues were toxic and weathered satisfactorily when used with a wetting agent alone or a wetting agent combined with lime, lime and bentonite, mineral oil, and lime and mineral oil; but not when combined with nicotine sulfate and bentonite. In other tests, not shown in the tables, performance was satisfactory when this chemical was used with lead arsenate; but with bentonite alone inactivation occurred. 2,4-diaminodiphenylamine, when present in toxic quantities, stains the conidia of *Sclerotinia fructicola* and *Glomerella cingulata* a deep brown. This staining of the conidia facilitates observations on toxicity and also is an indicator of the amount of the chemical present in the residue. When the chemical is combined with bentonite or with nicotine sulfate and bentonite, its typical color is seen only on the particles of floc formed by the physical contact of the materials, and the union appears so tight that none of the chemical is absorbed by the conidia. This appears not to occur when materials such as lime, lime-bentonite floc, or mineral oil are used with the chemical.

TABLE 1.—*Fungicidal effect of aqueous solutions of 2,4-diaminodiphenylamine, diphenylamine, and copper sulfate on conidia of Sclerotinia fructicola and Glomerella cingulata after various periods of perfusion*

Material			Conidia of *S. fructicola*		Conidia of *G. cingulata*	
Name	Approximate concentration	Period of perfusion	Appearance at end of incubation	Subsequent germination [3]	Appearance at end of incubation	Subsequent germination
	P p. m	*Hours*		*Percent*		*Percent*
2,4-diaminodiphenyl-amine	278	2	All appeared injured	0	All appeared injured	0
		4	do	0	do	0
		6	do	0	do	0
		22	do	0	do	0
		24	do	0	do	0
Diphenylamine [2]	11	2	Slight injury, no germination	100	No injury, no germination	100
		4	Slight injury, few buds	86	do	100
		6	do	22	do	100
		22	All appeared injured	0	All appeared injured	21
		24	do	0	do	0
Copper sulfate (calculated as Cu)	1.50	2	No apparent injury	100	No apparent injury	100
		4	do	85	do	100
		6	Slight injury apparent	18	Slight injury apparent	68
		22	All appeared injured	Trace	Many appeared injured	12
		24	do	Trace	do	50

[1] Transferred to oxidized potato juice 4-percent agar, incubated for 24 hours.
[2] 2 gm. of material added to 2,000 ml. of distilled H_2O
[3] About the solubility of copper in weathered alkaline bordeaux mixtures.

In these tests of relatively short duration, 2,4-diaminodiphenylamine proved to be effective and comparable with bordeaux mixture in toxicity to the conidia of the two fungi employed. But in none of these tests were the residues subjected to heavy continuous rains, and the data give no clue to what effect extended weathering would have had on toxicity. The high solubility of 2,4-diaminodiphenylamine should be detrimental to its effectiveness under conditions of longer exposure or to excessively heavy precipitation. In some field tests, not recorded in the tables, and conducted where such conditions existed, this was found to be true.

TABLE 2.—*Effect of weathering on the fungicidal activity of residues from 2,4-diaminodiphenylamine plus various adjuvants and from bordeaux mixture sprayed on glass cover slips on conidia of Sclerotinia fructicola and Glomerella cingulata* [1]

Combination	Period weathered in orchard (Days)	Precipitation total (Inches)	Conidia of S. fructicola after 24 hours—		Conidia of G. cingulata after 24 hours—	
			In Van Tieghem cell	On potato agar [2]	In Van Tieghem cell	On potato agar [2]
2,4-diaminodiphenylamine (2 lb.)	0	0	All appeared injured	No germination	All appeared injured	No germination.
+hydrated lime (8 lb.)	2	.42	All appeared plasmolyzed	do	All appeared plasmolyzed	Do.
+lead arsenate (2 lb.)	4	.62	do	do	do	Do.
+wetting agent 3 (15 gm)	7	.62	do	do	do	Do.
+water (100 gal.)	9	.62	do	do	do	Do.
	11	.95	do	do	do	Do.
2,4-diaminodiphenylamine (2 lb.)	0	.48	do	do	do	Do.
+hydrated lime (8 lb.)	2	.48	do	do	do	Do.
+bentonite (2 lb.)	4	.48	do	do	do	Do.
+lead arsenate (2 lb.)	7	1.06	do	do	do	Do.
+wetting agent 3 (15 gm.)	9	1.06	do	do	Many appeared normal	Many germinated.
+water (100 gal.)	11		do	do	do	Moderate germination.
2,4-diaminodiphenylamine (2 lb.)	0	.48	No apparent injury	Many germinated	46 percent germinated	Many germinated.
+bentonite (5 lb.)	2	.48	do	do	24 percent germinated	Do.
+nicotine sulfate (1 pt.)	4	.48	do	do	All germinated	Do.
+water (100 gal.)	7	1.06	do	do	do	Do.
	9	1.06	do	do	do	Do.
	11		do	do	do	Many germinated.
Copper sulfate (8 lb.)	0		All appeared injured	No germination	All appeared injured	No germination.
+hydrated lime (8 lb)	2	.42	All appeared plasmolyzed	do	All appeared plasmolyzed	Do.
+lead arsenate (2 lb.)	4	.62	Some appeared injured; some appeared not injured	Moderate germination	No apparent injury	Many germinated.
+water (100 gal.)	7	.95	All appeared plasmolyzed	No germination	Few appeared injured	Moderate germination.
	9		do	do	12 percent with buds	Many germinated.
	11		do	do	Few budded	Do.
2,4-diaminodiphenylamine (2 lb.)	0	0	All appeared plasmolyzed	No germination	All appeared injured	No germination.
+wetting agent 3 (15 gm.)	2	0	do	do	do	Do.
+water (100 gal.)	4	0	do	do	do	Do.
	7	0	do	do	do	Do.
	9		do	do	do	Do.
	11	2.28	do	do	do	Do.
2,4-diaminodiphenylamine (2 lb.)	0		All appeared plasmolyzed	No germination	All appeared plasmolyzed	No germination.
+mineral oil (1 qt.)	2	.18	do	do	do	Do.
+wetting agent 3 (15gm.)	4	.18	do	do	do	Do.
+water (100 gal.)	6	.18	do	do	do	Do.
	8	1.18	do	do	do	Do.
	10	1.33	do	do	do	Do.

2,4-diaminodiphenylamine (2 lb.)	0		do	do.	do.	Do.
+hydrated lime (8 lb.)	2	0	do	do.	do.	Do.
+wetting agent (15 gm.)	4	Trace	do.	do.	do.	Do.
+water (100 gal.)	6	Trace	do.	do.	do.	Do.
	8	Trace	do.	do.	do.	Do.
	10	46	do.	do.	do.	Do.
2,4-diaminodiphenylamine (2 lb.)	0		do.	do.	do.	Do.
+hydrated lime (8 lb.)	1	.93	do.	do.	do.	Do.
+mineral oil (1 qt.)	3	.93	do.	do.	do.	Do.
+wetting agent [2] (15 gm.)	5	.93	do.	do.	do.	Do.
+water (100 gal.)	7	1.76	do.	do.	do.	Do.
	12	2.18	do.	do.	do.	Do.

[1] All untreated conidia of both species had grown after the 24-hour incubation period in the Van Tieghem cells.

[2] Transferred to potato agar at the end of the 24-hour incubation period in the Van Tieghem cells. (See table 1.)

Santomerse (butylated phenylphenol sodium sulfonate).

Tables 3, 4, and 5 show data from limited field tests of 2,4-diamino-diphenylamine on apple and peach, conducted during the 1939 and 1940 seasons in young orchards on the grounds of the United States Horticultural Station, Beltsville, Md. The 1939 experiments on apple, as shown in table 3, were more reliable than those conducted during 1940, because of the greater number of apples examined. In 1939 scab was well controlled on all varieties and russet was very light, even on the fairly sensitive York Imperial variety. In the 1940 tests the crop was light, but in all cases a considerable reduction of fruit and leaf scab (tables 3 and 4) was effected by the use of 2,4-diaminodiphenylamine combined with lime or with lime and oil. In 1940 russeting was serious even on nonsprayed trees of sensitive varieties, owing to the cold spring. Under these conditions, 2,4-diaminodiphenylamine compared favorably with but was not better than the standard treatments.

TABLE 3.—*Effect of 6 applications of 2,4-diaminodiphenylamine spray combinations on scab and russet on fruits of various apple varieties*

Year and combination	Variety	Fruits examined	Fruits russeted	Fruits scabbed
1939		*Number*	*Percent*	*Percent*
2,4-diaminodiphenylamine (2 lb) +hydrated lime (8 lb.) +bentonite (2 lb) +lead arsenate (2 lb) +wetting agent [2] (15 gm) +water (100 gal.)	Starking Rome Beauty York Imperial.	228 645 75	1 3 1. 9 [1] 26. 6	3 5 Trace 0
Untreated	Starking	374	0	83 0
1940				
2,4-diaminodiphenylamine (2 lb.) +hydrated lime (8 lb.) +lead arsenate (2 lb.) +mineral oil (1 qt.) +wetting agent [2] (15 gm) +water (100 gal.)	Delicious Stayman Winesap Grimes Golden Jonathan Golden Delicious York Imperial	9 6 4 54 11 43	0 0 100. 0 22 2 100 0 14. 0	11. 0 0 0 0 0 2. 3
Untreated	Delicious Stayman Winesap Grimes Golden Jonathan Golden Delicious	8 87 171 61 11	12. 5 2 3 11. 6 80 0 100. 0	100. 0 86 2 38 0 64. 0 36. 3

[1] Light.
[2] Santomerse (butylated phenylphenol sodium sulfonate).

TABLE 4.—*Effect of 6 applications of 2,4-diaminodiphenylamine spray combinations and of other sprays on scab on leaves of various apple varieties, at harvest, 1940*

[100 leaves examined from each of 3 parts of each tree; "untreated" data based on 4 trees and other data on 1 tree]

Combinaton	Leaves infected on—							
	5-year-old Delicious	4-year-old Delicious	5-year-old Stayman Winesap	4-year-old Stayman Winesap	5-year-old Grimes Golden	5-year-old Jonathan	5-year-old York Imperial	5-year-old Golden Delicious
2,4-diaminodiphenylamine (2 lb) +hydrated lime (8 lb.) +lead arsenate (2 lb) +wetting agent [1] (15 gm.) +water (100 gal)	47.0	Trace	12.0	Trace	Trace	Trace	Trace	0
2,4-diaminodiphenylamine (2 lb) +hydrated lime (8 lb.) +lead arsenate (2 lb) +wetting agent [1] (15 gm.) +mineral oil (1 qt.) +water (100 gal.)	5 0	Trace	Trace	Trace	Trace	Trace	0	0
Carbon sulfur (8 lb.) +lead arsenate (2 lb) +lime (8 lb). +water (100 gal)	25.0	Trace	Trace	Trace	Trace	Trace	Trace	0
Line and sulfur combinations [2]	Trace	Trace	Trace	Trace	Trace	Trace	Trace	0
Untreated	89.5	75.5	82.6	67 0	72 0	42 0	44 8	32.0

[1] Santomerse (butylated phenylphenol sodium sulfonate).
[2] Liquid lime-sulfur 2–100 pre-pink; lime-sulfur plus lead arsenate 2–2–100—pink and calyx; wettable sulfur, lime, and lead arsenate 8–4–2–100—3 cover applications.

TABLE 5.— *Effect of 5 applications of 2,4-diaminodiphenylamine spray combinations on scab and brown rot of peach fruit*

Year and combination	Variety	Trees	Fruits examined	Scab		Fruit affected by brown rot [2]
				Severity on affected fruit [1]	Fruit affected	
1939		*Number*	*Number*		*Percent*	*Percent*
2,4-diaminodiphenylamine (2 lb.) +hydrated lime (8 lb) +bentonite (2 lb) +wetting agent [3] (15 gm.) +water (100 gal)	Alexander	1	108	Trace		37 9
	Carman	1	49	Light	100 0	32.7
	Elberta	1	45	do	100.0	0
Untreated	Alexander	3	430	Moderate	90 0	99.0
	Carman	1	66	Severe	100 0	36.4
	Elberta	6	245	do	100.0	8 1
1940 [4]						
2,4-diaminodiphenylamine (2 lb) +hydrated lime (8 lb) +wetting agent [3] (15 gm) +water (100 gal)	Alexander	1	258	Light	2 8	1.6
	Carman	1	248	do	5 0	22.6
	Elberta	3	689	do	13 8	6.3
Untreated	Alexander	3	660	Moderate	85 0	42 0
	Carman	3	279	do	71.0	33.3
	Elberta	12	2,510	do	100 0	25.3

[1] Light scab, 1 or 2 spots; moderate scab, several to a dozen spots; severe scab, many spots.
[2] Poor control of insects in check trees, resulting in spread of brown rot.
[3] Santomerse (butylated phenylphenol sodium sulfonate).
[4] Lead arsenate plus zinc-lime added to shuck and first cover sprays to control worms.

In the peach tests, as implied in table 5, the crop of fruit was light in 1939. Curculio and oriental peach moth injury were very serious during both seasons because of the nearness of the treated trees to the unsprayed check trees, and brown rot was more of a factor than usual because these insects produce portals of entry for the brown rot pathogen. In the 1939 tests zinc-lime plus lead arsenate was not added to the 2,4-diaminodiphenylamine sprays, whereas it was added in 1940.

Under the conditions of the peach tests, although the treated trees were flanked on each side with untreated trees, 2,4-diaminodiphenylamine gave a very fine performance against scab and brown rot. Whenever scab was present the lesions were small and scanty, and none of the fruit could be classed as culls because of it. The tests were limited, however, and give only a clue to the behavior of this material against the organisms that cause scab and brown rot under more rigid conditions.

On cherry varieties, tests were conducted with 2,4-diaminodiphenylamine during 1939 and 1940 on Montmorency, Early Richmond, and Saint Medard. In both seasons the material, though protecting against leaf spot when applied frequently, gave no promise of being a protectant over a long period, in which respect it did not compare with bordeaux mixture or with copper phosphate mixture. In both seasons control was good up to the time of the post-harvest application, but leaf spot became severe during the long period following this application, indicating a deficiency in the lasting qualities of the residues of the compound.

FUNGICIDAL AND PHYTOCIDAL PROPERTIES OF SOME CLOSELY RELATED DIPHENYLAMINES

Table 6 shows the relative fungicidal value of 2,4-diaminodiphenylamine and some closely related diphenylamines. Since none of the true isomers of the 2,4-diaminodiphenylamine were available for the tests, the relative value of these will have to await their preparation. Of the closely related compounds, 2',4'-diamino-2-phenyldiphenylamine and diphenylamine killed the largest percentage of conidia of both *Sclerotinia fructicola* and *Glomerella cingulata*. 4-nitrodiphenylamine, 4-chlorodiphenylamine, and diphenylparaphenylene diamine were toxic to the conidia of *S. fructicola* in varying degrees but not to those of *G. cingulata*. The rest of the compounds exhibited little or no toxicity. It is interesting to note that the 4 position appears to be correlated with toxicity provided no other position is occupied by a dissimilar group. With 2,4-diaminodiphenylamine, both positions are occupied by a similar group.

TABLE 6.—*Fungicidal effect of perfusion in saturated solutions of 2,4-diaminodiphenylamine and of some closely related diphenylamines on the conidia of Sclerotinia fructicola and Glomerella cingulata*

Compound	Structural formula	Solubility in water	Conidia of indicated fungus killed in—									
			2 hours		4 hours		6 hours		22 hours		24 hours	
			Sclero-tinia	Glo-merella	Sclero-tinia	Glo-merella	Sclero-tinia	Glo-merella	Sclero-tinia	Glo-merella	Sclero-tinia	Glo-merella
2,4-diaminodiphenylamine	H_2N– ring –N(H)– ring –NH_2	P.p.m 278	Percent 100	Percent 100	Percent 100	Percent 100	Percent 100	Percent 100	Percent 100	Percent 100	Percent 100	Percent 100
Diphenylamine	ring –N(H)– ring	11	0	0	14	0	72	0	100	79	100	100
Phenothiazine	ring –N(H)– / S ring	12	0	0	0	0	0	0	0	0	0	0
2,4-dinitrodiphenylamine	O_2N– ring –N(H)– ring –NO_2	206	0	0	0	0	0	0	0	0	0	0
4-nitrodiphenylamine	O_2N– ring –N(H)– ring	Trace	0	0	0	0	0	0	99+	0	100	0
2-nitrodiphenylamine	ring –N(H)– ring –NO_2	3	0	0	0	0	0	0	0	0	0	0

TABLE 6.—*Fungicidal effect of perfusion in saturated solutions of 2,4-diaminodiphenylamine and of some closely related diphenylamines on the conidia of Sclerotinia fructicola and Glomerella cingulata*—Continued

Compound	Structural formula	Solubility in water	Conidia of indicated fungus killed in—									
			2 hours		4 hours		6 hours		22 hours		24 hours	
			Sclero-tinia	Glo-merella	Sclero-tinia	Glo-merella	Sclero-tinia	Glo-merella	Sclero-tinia	Glo-merella	Sclero-tinia	Glo-merella
		P. p. m.	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
4'-nitro-4-methyldiphenylamine		3	0	0	0	0	0	0	0	0	0	0
2',4'-diamino-2-phenyldiphenyl-amine		67	39	0	54	0	42	54	74	42	86	45
Dipicrylamine		21	0	0	0	0	0	0	0	0	4	0
4-chlorodiphenylamine		8	0	0	0	0	0	0	49	0	88	0
4'-chlorodiphenylamine-2-carboxylic acid		20	0	0	0	0	0	0	0	0	0	0
3',5',3,5-tetrachlorodiphenylamine		14	0	0	0	0	0	0	0	0	0	0
2'-chlorodiphenylamine-2-carboxylic acid		14	0	0	0	0	0	0	0	0	0	0

Compound										
3'-chlorodiphenylamine-2-carboxylic acid	0	12	0	4	0	0	0	0	0	6
3',5',3,5-tetrabromodiphenylamine	0	4	0	0	0	0	0	0	0	5
2'-methyldiphenylamine-2-carboxylic acid	0	0	0	0	0	0	0	0	0	10
4'-methyldiphenylamine-2-carboxylic acid	0	0	0	0	0	0	0	0	0	5
3'-methyldiphenylamine-2-carboxylic acid	6	10	6	14	9	6	0	0	0	9
Diphenylparaphenylene diamine	0	41	0	34	0	0	0	0	0	4
Phenothiazine-N-carboxylic acid chloride	0	0	0	0	0	0	0	0	0	13

Diphenylamine and 2',4'-diamino-2-phenyldiphenylamine were the only members of the tested group that were phytocidal. All of these compounds were sprayed on Red Kidney bean plants, and injury was caused only by the two just mentioned. When sprayed on leaves and fruits of peach, cherry, plum, apricot, pear, apple, and quince, 2,4-diaminodiphenylamine in combination with a wetting agent, lime, lead arsenate, lime and bentonite, mineral oil, mineral oil plus lime, and nicotine sulfate plus bentonite caused no injury.

SUMMARY AND CONCLUSIONS

Perfusion, spray-residue, and field tests, conducted during a period of several years, showed that 2,4-diaminodiphenylamine possesses promising fungicidal properties and does not cause injury to apple, pear, peach, plum, cherry, apricot, quince, or bean.

2,4-diaminodiphenylamine is basic in character and is compatible with lime, bentonite plus lime, lead arsenate, and mineral oil. When combined with bentonite or with bentonite flocculated with nicotine sulfate, its toxicity is lost.

2,4-diaminodiphenylamine is difficult to wet in water but is readily dispersed by the addition of a small amount of a wetting agent.

The fungicidal properties of saturated solutions of a series of closely related diphenylamines were also tested. Diphenylamine, one of the parent materials of 2,4-diaminodiphenylamine, and 2',4'-diamino-2-phenyldiphenylamine approached 2,4-diaminodiphenylamine in toxicity. 4-nitrodiphenylamine, 4-chlorodiphenylamine, and diphenyl-paraphenylene diamine were toxic to the conidia of *Sclerotinia fructicola* but not to those of *Glomerella çingulata*. With the exception of diphenylamine and 2',4'-diamino-2-phenyldiphenylamine, none of the compounds tested were injurious to Red Kidney bean plants.

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INHERITANCE OF CROSS- AND SELF-STERILITY AND SELF-FERTILITY IN BETA VULGARIS [1]

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INTRODUCTION

Difficulty in securing self-fertilized seed from cultivated varieties of *Beta vulgaris* L. has been commonly experienced. Self-fertility, which contrasts strikingly with the more common self-sterile condition, is now becoming familiar to most breeders of sugar beets and garden beets. Various self-sterile types and a limited number of self-fertile types of sugar beets were collected by the writer, with emphasis upon selections from curly top resistant varieties. Genetic studies of cross-sterility, self-sterility, and self-fertility were conducted upon selected parental material having clearly defined differences, careful account being taken of variability caused by environmental factors.

REVIEW OF LITERATURE AND THEORY

Papers dealing with sterility in *Beta vulgaris* have been written by Archimovitch (*1*),[3] Brewbaker (*3*), Briem (*4*), Down and Lavis (*8*), Hallqvist (*15*), Kohls (*20*), Magruder (*23*), Munerati (*24*), Raleigh (*26*), Stewart (*28*), and Sundelin (*30*). Self-fertile types of beets have been described by Archimovitch (*1*), Kharechko-Savitskaîa,[4] Magruder (*23*), Stewart, Lavis, and Coons (*27*), and Sundelin (*30*). The basis of inheritance of self-sterility and self-fertility in *Beta vulgaris* has apparently received but little attention.

It will be seen that instances of sterility behavior in *Beta vulgaris* obtained by the writer are somewhat complex but are fairly well explained by certain modifications of the oppositional hypothesis (*10, 13*). The oppositional hypothesis supposes the presence in the style of substances that produce incompatibility when a pollen tube enters with an S factor in common with stylar tissue. An S^1S^2 plant is sterile or incompatible with all its own S^1 and S^2 pollen, and with S^1 and S^2 pollen from any other source, but fertile with S^3 to S^n pollen, S^1 to S^n representing an allelic series. Crosses of the type $S^1 S^2 \times S^3 S^4$ yield four classes, $S^1S^3 + S^1S^4 + S^2S^3 + S^2S^4$. Cross-sterility in both

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directions between all plants within any one of these four classes is expected, but crosses between different classes should be fertile. A cross of the type $S^1S^2 \times S^2S^3$ produces two classes, $S^1S^3 + S^2S^3$, one of which is identical with the pollen parent.

The factor S' for self-fertility (*9, 12*), allelic to the sterility factors S^1 to S^n, is exceptional and does not produce self-sterility. Heterozygous $S'S^a$ plants upon selfing are expected to produce only self-fertile offspring ($S'S'$ and $S'S^a$) without recovery of the S^aS^a genotype because of sterility to S^a pollen.

With a single series of oppositional factors, self-sterile plants are normally heterozygous for S factors. Consequently, if a cross is fertile in one direction, it is expected to be fertile in the other direction. Nonreciprocal behavior is expected only after the unusual selfing of self-sterile plants. Selfing a self-sterile plant, S^1S^2, may yield three classes, $S^1S^1 + 2\ S^1S^2 + S^2S^2$, with nonreciprocal sterility between the first and second and between the third and second classes.

Although nonreciprocal cross-sterility is not commonly expected with a single series of oppositional factors, Lawrence (*21*) has shown that nonreciprocal behavior may commonly result with duplicate oppositional factors, a condition expected with polyploidy. This theory seems to fit certain results to be described with beets, although there is little reason to expect polyploidy with the $2n = 18$ chromosome condition (*2*) in *Beta vulgaris*. Lawrence's theory of duplicate factors assumes that duplicate or identical genes are carried by two different chromosomes. The duplicate of S^1 will be designated Z^1, the genes being identical but carried by different chromosomes. Confusion because of failure to consider S^1 and Z^1 identical has been experienced (*22, 29*). It is assumed that a single S or Z factor carried by the pollen and not present in stigmatic tissue produces fertility, hence an $S^1S^1Z^1Z^2$ female should be fertile with S^1Z^3 or S^3Z^2 pollen. The behavior of the duplicate factors will be illustrated further as results are interpreted.

EXPERIMENTAL PROCEDURE

MATERIAL

One of the early curly top resistant strains produced by Carsner (*6*) and known as 12c proved to be of interest because of a strong degree of cross-sterility. After preliminary studies from closely bred material within the 12c strain, three plants, designated clones 1, 2, and 4, were selected in 1931 for intensive studies. Self-fertility arose from selfing the self-sterile clone 4, presumably by mutation. Another of the original curly top resistant strains, known as 286 (*6*), segregated for self-fertility, and strain 1167, selected from strain 286 in 1931, was found to carry the genetic factor S' for self-fertility. Carsner (*6*) had done considerable inbreeding in developing the 286 strain from which 1167 was derived, and the 12c strain was also obtained by relatively close breeding Self-sterile plants were found to predominate greatly in the curly top resistant variety U. S. 1 (*7*), and a few individuals whose degree of self-sterility was known from extensive experience were propagated vegetatively for genetic investigations.

Some of the extensive selections made by Tracy,[5] without regard to curly top resistance, were tested for self-sterility and self-fertility.

Some of these selections were rather strongly self-sterile, but the Tracy strain 2769 was found to segregate for self-fertility, and a selection, designated clone 70, was propagated vegetatively and utilized for genetic studies.

METHODS

It was recognized at the beginning of the work in 1930 that a study of self-sterility and self-fertility in beets would require vegetative propagation to carry critical material along for perhaps several years. Fortunately, the task of building vegetative clones was not difficult, because succulent cuttings from vegetative seedstalks took root readily and clones were easily carried along from year to year with a moderate degree of attention. The most critical factor was the matter of inducing seedstalks to become vegetative, to insure vigorous cuttings. This was accomplished by controlling light and temperature in a manner described by Owen, Carsner and Stout (*25*).

Paper bags made of several kinds of material were used in testing compatibility. The most suitable were those made from ordinary 30-pound parchment butter wrappers, 8½ × 11 inches. These wrappers were folded and glued by hand; then a strip of varnish was painted over the seams to prevent moisture from interfering with the glue. Lightweight kraft bags of various sizes also proved useful, but for sterility studies there is considerable advantage in having a white and partly translucent bag so that the pollen can be readily seen. A large bag is usually not necessary to detect sterility relationships. To determine self-fertility or self-sterility of the blossoms, the paper bags were placed on the branches prior to flowering. Crosses were made by exchanging paper bags at the proper time, and with self-sterile plants as female parents there was no need for emasculation.

It was useful to know that beet pollen can be stored at low temperatures. Kharechko-Savitskafa (*18*) reports germination from beet pollen stored for 49 days at low temperature and low humidity. The writer stored pollen for various periods in an electric refrigerator at approximately 36° F. and found some viable pollen even after 60 days. A week's storage has been considered entirely safe and was adopted whenever it was impossible to have plants flowering at the same time.

The bagging work was first done in the open field, but it soon became apparent that there were many advantages in using the greenhouse. Here insect and weather damage were largely eliminated, and by adjusting day length and temperature it was possible to keep plants in flower over a longer period.

RESULTS

HERITABLE AND ENVIRONMENTAL INFLUENCES COMPARED

Both environmental and heritable influences were observed to affect self-sterility in beets. An example of a heritable difference in degree of self-sterility is as follows: During the spring of 1932, branches from two vegetative clones selected from the U. S. 1 variety were bagged at flowering time. Clone 36, with 17 paper bags, did not produce a single seed, while clone 73 produced a total of 15 seeds in 17 bags (11 of the 17 bags produced no viable seeds). Further observation indicated that the difference between these two clones was consistent.

Other examples of differences in degree of self-sterility could be cited from experience with other vegetative clones. There appeared to be gradations up to occasional intermediate types that were sometimes difficult to distinguish from truly self-fertile types. Nevertheless, the truly self-fertile condition was clearly defined and was seldom confused with the common self-sterile condition that predominates in commercial varieties of sugar beets.

In the field, types with a self-sterile tendency, single-plant or single-clone isolations set seed more easily than parts of the same plants under paper bags. Evidently the paper bags interfered with fertilization to some extent, or with the development of the embryo. Clone 4 of the 12c strain and clone 86 of the Pioneer variety of sugar beets furnished examples of practically complete self-sterility in field isolations as well as under paper bags. With each of these clones a very small amount of the seed produced in favorable field isolations turned out to be chiefly red garden beet hybrids. Since no red beet or Swiss chard plants were observed to be flowering in the vicinity of the isolations, this contamination probably resulted from distant sources of pollen. Contamination was probably encouraged by the high degree of self-incompatibility, which caused the flowers to remain unfertilized and the stigmas receptive for a relatively long period. In other instances clones that were classed as self-sterile from tests with paper bags produced fairly good seed in field isolations. The best field results with self-fertilization were secured in the higher valleys of northern Utah (5,000 to 6,000 feet elevation), where temperatures were lower than in Salt Lake Valley (about 4,300 feet elevation).

A surprising amount of self-fertilized seed was set on some self-sterile plants in early summer near the end of each season's work in the greenhouse and after a prolonged period of flowering. Experience with clone 19, a selection from the 12c strain, was particularly conspicuous. For 2 years this clone was studied in connection with cross-sterility with other 12c plants. Results were confusing the first year (1931), but near the end of the next spring this clone produced self-fertilized seed in abundance in the greenhouse. A month earlier the same plants were strongly self-sterile, and a further test of new plants of the same clone in the field in 1932 again indicated complete self-sterility. A similar observation was made with the self-sterile clone 144 derived from the U. S. 1 variety. A quantity of self-fertilized seed was secured late in the season on an old plant after the clone had been studied for a period of 4 years and always observed to be strongly self-sterile. It was apparent after these experiences that classifications for self-fertility were not always reliable on old plants late in the season in the greenhouse, after a prolonged period of flowering.

It is perhaps significant that this unusual self-fertility was most noticeable at the end of the season in the greenhouse on plants that had passed through several flowering periods. The best flowers were usually produced with the first flowering, and under field conditions this is normally the final flowering. In the greenhouse, on the other hand, it is possible to cut off part of the inflorescence and, with proper growing conditions, produce new flowering branches. This process was sometimes repeated four or five times in a season, and self-fertility was most noticeable during the later periods. This resembles

the exceptional self-fertility at the end of the season that East and Park (*11*) have described in self-sterile species of *Nicotiana*. An explanation of this end-season fertility suggested by East and Park (*11*) is the disappearance from the style of substances that had previously been responsible for a chemical antagonism between the stylar tissue and pollen tubes.

NONRECIPROCAL CROSS-STERILITY IN THE 12C STRAIN

A special feature of the cross-sterility found in the 12c strain was a decided nonreciprocal behavior. Many of the observed results, including the nonreciprocal behavior, agree rather well with expected results, assuming duplicate oppositional factors (*21*) for sterility. This theory, therefore, will be described as data are presented, although experiments were not carried far enough to check certain crucial points.

Twenty plants from a closely bred selection from the 12c strain were grown in 1931, and an attempt was made to cross each plant in both directions with the other 19 plants. Three of the 20 plants were inclined to be sterile as females but fertile as pollen parents. Plant No. 4, later designated clone 4, was sterile to pollen from 16 of the 19 other plants, but when used as a pollen parent it was fertile in most combinations. Three plants, clones 1, 2, and 4, were selected for further study. The center of interest was the nonreciprocal cross-sterility among these 3 plants (fig. 1). Clone 4 was sterile to pollen from clones 1 and 2,

♂ / ♀	CLONE 1 $S^1S^1Z^1Z^2$	CLONE 2 $S^1S^2Z^2Z^3$	CLONE 4 $S^1S^2Z^2Z^4$
CLONE 1 $S^1S^1Z^1Z^2$	**S**	$S^1S^1Z^2Z^3$ $S^1S^2Z^1Z^3$ $S^1S^2Z^2Z^3$ $S^1S^1Z^1Z^3$	$S^1S^1Z^1Z^3$ $S^1S^2Z^1Z^4$ $S^1S^1Z^1Z^4$ $S^1S^1Z^2Z^3$ $S^1S^1Z^2Z^4$ $S^1S^2Z^1Z^3$ $S^1S^2Z^2Z^4$ $S^1S^2Z^2Z^3$
CLONE 2 $S^1S^2Z^2Z^3$	—	**S**	$S^1S^1Z^2Z^4$ $S^1S^2Z^3Z^4$ $S^1S^2Z^2Z^4$ $S^1S^2Z^2Z^4$
CLONE 4 $S^1S^2Z^2Z^4$	—	—	**S**

S SELF-STERILE   — CROSS-STERILE

FIGURE 1.—Explanation, on basis of duplicate factors, of nonreciprocal cross-sterility among clones 1, 2, and 4. Genotypes of parental clones shown in margins, and genotypes of all F_1 offspring shown for the three fertile combinations.

but clones 1 and 2 were fertile to pollen from clone 4. Clone 2 was sterile to pollen from clone 1, but clone 1 was fertile to pollen from clone 2. These results were clearly defined and were confirmed by means of repeated tests during the course of 4 years' work.

To explain the breeding behavior of clones 1, 2, and 4 on the basis of duplicate factors for sterility, good agreement between theory and most of the observations results if it is assumed that clone 1 was of constitution $S^1S^1Z^1Z^2$; clone 2, $S^1S^2Z^3Z^3$; and clone 4, $S^1S^2Z^3Z^4$. The

Z factors are assumed to be exact duplicates of the S factors but carried by different chromosomes; that is, S^1 is assumed to have exactly the same effect as Z^1, and S^2 the same as Z^2, etc. Pollen is assumed to be effective if it carries an allelomorph of either S or Z not present in stigmatic tissue. In accordance with this theory all possible F_1 genotypes for the three fertile combinations among clones 1, 2, and

♀ \ ♂	CLONES			F₁ PLANTS (CLONE 1 X CLONE 2)						
	$S^1S^1Z^1Z^2$ **1**	$S^1S^2Z^2Z^3$ **2**	$S^1S^2Z^2Z^4$ **4**	$S^1S^1Z^2Z^3$ — **A**	**B**	$S^1S^2Z^1Z^3$ — **C**	**D**	$S^1S^2Z^2Z^3$ **E**	$S^1S^1Z^1Z^2$ **F**	**G**
1 $S^1S^1Z^1Z^2$	S	+	+	+	+	+	+	+	+	+
2 $S^1S^2Z^2Z^3$	−	S	+	−	−	−	−	−	−	−
4 $S^1S^2Z^2Z^4$	−	−	S	−	−	−	−	−	−	−
A $S^1S^1Z^2Z^3$	−	−	+	S	−	−	−	−	−	−
B $S^1S^2Z^1Z^3$	−	−	+	−	S	−	−	−	s	−
C $S^1S^2Z^1Z^3$	−	−	+	−	−	S	−	−	−	−
D $S^1S^2Z^2Z^3$	−	−	+	−	−	−	S	−	−	s
E	−	−	+	−	−	−	−	S	−	s
F $S^1S^1Z^1Z^2$	+	+	+	+	+	+	+	+	S	s
G	+	+	+	f	+	+	+	f	s	S

OBSERVED AND EXPECTED   EXPECTED BUT NO OBSERVATION

+ FERTILE   F FERTILE
− STERILE   S STERILE
S SELF-STERILE

FIGURE 2.—Observed results and explanation, on basis of duplicate factors, of cross-sterility among F_1 plants from clone 1 × clone 2 and with parental clones 1, 2, and 4.

4 are indicated in figure 1. For the F_1 population derived from clone 2 × clone 4, the genotype $S^1S^2Z^3Z^4$ occurs twice because it is formed in two different ways.

The reactions of the F_1 plants from clone 1 × clone 2 are shown in figure 2. Both theory and observation indicate 2 distinct classes of F_1 plants. According to the theory, 3 genotypes, $S^1S^1Z^2Z^3$, $S^1S^2Z^1Z^3$, and $S^1S^2Z^2Z^3$, carry 3 kinds of genes (S^1 or Z^1, S^2 or Z^2, and S^3 or Z^3)

and are expected to be sterile to pollen from clones 1 and 2 and all of the F₁ plants. Another class of plants, consisting of 1 genotype, $S^1S^1Z^1Z^3$, carries only 2 kinds of genes, and, failing to carry S^2 or Z^2, it is expected to be fertile with half of the pollen from clones 1 and 2 as well as with three-fourths of the pollen from clone 4 and all F₁ plants except those within its own class. The theory requires a 3 : 1

♀ \ ♂	CLONES 1	CLONES 2	CLONES 4	A	B	C	D	E	F	G	H	I	J
Clone 1	S	+	+	F	+	F	+	+	+	+	+	+	+
Clone 2	−	S	+	F	F	+	+	+	F	+	+	+	+
Clone 4	−	−	S	−	−	−	−	−	−	−	−	−	−
A	F	F	+	S	s	F	F	F	F	F	F	F	F
B	F	F	+	s	S	F	F	F	F	+	+	+	F
C	F	+	+	F	F	S	s	F	F	F	F	F	+
D	+	+	+	F	F	s	S	F	+	+	+	F	+
E	−	−	−	s	s	s	s	S	s	s	s	s	s
F	−	−	−	−	s	s	−	s	S	−	−	−	−
G	−	−	−	s	−	−	−	s	−	S	−	−	−
H	−	−	−	s	−	s	−	s	−	−	S	−	−
I	−	−	−	s	−	s	−	s	−	−	−	S	−
J	−	−	−	s	s	−	−	s	−	−	−	−	S

(Header spans: columns 1, 2, 4 = CLONES; columns A–J = F₁ PLANTS (CLONE 2 X CLONE 4). Clone genotypes: 1 = $S^1S^1Z^1Z^1$, 2 = $S^2S^2Z^2Z^2$, 4 = $S^1S^2Z^1Z^2$.)

OBSERVED AND EXPECTED   EXPECTED BUT NO OBSERVATION

+ FERTILE   F FERTILE
− STERILE   S STERILE
S SELF-STERILE

FIGURE 3.—Observed results and explanation, on basis of duplicate factors, of cross-sterility among F₁ plants from clone 2 × clone 4 and with the parental clones 1, 2, and 4.

ratio for these 2 classes of plants, while 5 plants (A to E) were observed in the first class and 2 plants (F and G) were observed in the second class. Unfortunately, this theory, which agrees so well with observed results, was not in mind when the sterility reactions were being tested, and critical data regarding sterility between plants F and G (fig. 2) were not recorded. The F₁ plants, clone 1 × clone 2, were also segregated for male sterility. Of 60 plants observed, 40 produced normal

pollen and 20 produced partly or completely aborted pollen. Only plants that produced normal pollen were used in the studies indicated in figure 2.

All observed results agree perfectly with the expected sterility and fertility reactions from the F_1 plants, clone 2 × clone 4 (fig. 3), but several gaps occur in the observations. Half of the F_1 plants, or the

♀ \ ♂	CLONES 1	2	4	F₁ PLANTS (CLONE 1 × CLONE 4) A	B	C	D	E	F	G	H	I	J
CLONES 1	S	+	+	+	+	+	+	+	+	+	+	+	+
2	−	S	+	+		+		+	+		+	+	+
4	−	−	S	−	−	−	−	−	−	−	−	−	−
A	+	+	+	S	+		+	+			+	+	+
B	+		+	+	S	+		+	+		+	+	+
C	+		+	+	+	S	+	+	+		+		
D		+	+	+			S	+	+				+
E	−		+	+	+	+		S	+		+	+	+
F	−	+	+	+	+	+	−	S			+	+	+
G	−		+	+				−		S		+	+
H	−		+	+	+			+	+	+	S		+
I	−	−	−	−	−	−	−	−	−	−		S	
J	−		−	−				−				−	S

+ FERTILE
− STERILE
S SELF-STERILE

FIGURE 4.—Observed results of cross-sterility among F_1 plants of clone 1 × clone 4 and with parental clones 1, 2, and 4.

genotypes $S^1S^1Z^3Z^4$ and $S^2S^2Z^3Z^4$, are expected to be fertile as females in all combinations, except in crosses between identical genotypes, and half ($S^1S^2Z^3Z^4$), formed in two different ways (fig. 1), are expected to be sterile as females in all combinations. Figure 3 shows four plants (A to D) that appear to belong in the first class and six plants (E to J) that appear to belong in the second class.

Reactions with clone 4 and F_1 plants, clone 2 × clone 4, were tested further with additional plants grown in the field. An abundance of

pollen, collected in paper bags from 45 F_1 plants, was mixed and applied to branches of clone 4 previously protected from foreign pollen by paper bags. The results were all negative, indicating complete cross-sterility of clone 4 with pollen from all of the 45 plants in the F_1 population. Pollen from clone 4 was also applied to the 45 F_1 plants, and results may be added to those for the 10 plants indicated in figure 3. This makes a total of 55 F_1 plants tested, of which 29 were fertile and 26 were sterile to the pollen from clone 4. This is a good $1:1$ ratio and agrees with expected results.

Some of the observed results (fig. 4) with the F_1 plants, clone 1 × clone 4, do not agree with what is expected from the theoretical considerations. The eight classes of F_1 genotypes ($S^1S^1Z^1Z^3$, $S^1S^1Z^1Z^4$, $S^1S^1Z^2Z^4$, $S^1S^2Z^2Z^4$, $S^1S^2Z^1Z^4$, $S^1S^1Z^2Z^3$, $S^1S^2Z^1Z^3$, and $S^1S Z^2Z^3$) expected from clone 1 × clone 4 are indicated in figure 1. The genes Z^3 and Z^4 do not occur together in any one of these eight genotypes, hence all F_1 plants should be fertile to pollen from clone 4 ($S^1S^2Z^3Z^4$). Observed results with the F_1 plants I and J (fig. 4), which show sterility to clone 4 pollen, are, therefore, not in agreement with the expected results. It can be shown that the theory does not explain the behavior of the F_1 plant I (fig. 4), which was tested in seven of the nine possible combinations with other F_1 plants and was sterile as a female in all combinations for which it was tested. Another disagreement with expected results is the consistently positive reaction of clone 2 with pollen from F_1 plants when a $1:1$ ratio of positive to negative reactions is expected.

Other observed results with the F_1 population, clone 1 × clone 4, are in agreement with the theory of duplicate factors under consideration. The reactions with plant F as a female (fig. 4) are particularly indicative of what is expected. If this plant were of constitution $S^1S^1Z^2Z^4$, $S^1S^2Z^2Z^4$, or $S^1S^2Z^1Z^4$, the absence of the Z^3 gene would explain the negative reaction to pollen from clone 1 ($S^1S^1Z^1Z^2$) and the positive reaction to pollen from both clone 2 ($S^1S^2Z^3Z^3$) and clone 4 ($S^1S^2Z^3Z^4$). If plants A to D (fig. 4) were of constitution $S^1S^1Z^1Z^3$ or $S^1S^1Z^1Z^4$, their consistently positive reactions as females with pollen from clones 1, 2, and 4 and with most of the F_1 plants could be explained.

The experiments were not carried far enough to constitute a critical check on the theory of duplicate factors, but in general the agreement between theoretical and observed results is sufficiently close to warrant careful consideration of this theory in any future work that might be attempted. Reactions among the three parental clones 1, 2, and 4, and results with F_1 plants, clone 1 × clone 2, and clone 2 × clone 4, agree remarkably well with expected results on the basis of duplicate factors for cross-sterility. The possibility of modifying genes responsible for lack of agreement between observed and expected results with the F_1 plants, clone 1 × clone 4, may be considered, but data are too limited to postulate the precise behavior of such modifiers.

INHERITANCE OF SELF-FERTILITY

It will be shown that self-fertility in beets may be determined by a single genetic factor designated S'. Although the inheritance of self-fertility is well explained by this single factor, the allelomorphs of S', expected to determine cross-sterility reactions, could not be identified. The evidence clearly indicates the presence of auxiliary factors for

cross-sterility in addition to allelomorphs of the factor S'. The exact nature of these auxiliary factors is unknown, but one series of backcrosses gave results in agreement with expectation, assuming a duplicate series of Z factors in addition to the S series. In another series of backcrosses the results failed to agree entirely with expectation based upon duplicate factors.

The designation for homozygous self-fertile plants will be $S'S'$, and for heterozygous self-fertile plants $S'S^a$ (or $S'S^b$ or $S'S^x$). For convenience all self-sterile plants are arbitrarily designated S^aS^b, although in a group of self-sterile plants many different S allelomorphs may be involved. In general, crosses between self-sterile plants and heterozygous self-fertile plants will be designated $S^aS^b \times S'S^x$. Here S^x in relation to S^a and S^b is unknown but, without proof to the contrary, it may be assumed in each case to be different from S^a or S^b. When necessary to consider duplicate factors for cross-sterility, different S and Z factors are distinguished by numerical superscripts, with $S^1S^2Z^3Z^4$, etc., representing self-sterile plants and $S'S^2Z^3Z^4$, etc., representing heterozygous self-fertile plants with the factor for self-fertility limited to the S series. The allelomorph S', responsible for self-fertility, is the only factor in the S series whose identity is believed to be established; hence, all other designations are purely arbitrary.

In hybrids to be described, the factor S' for self-fertility came either from the curly top resistant strain 1167 or from clone 70, a selection from the Tracy strain 2769. The self-sterile clones 90 and 144, used in backcross studies, were derived from the curly top resistant variety U. S. 1.

TABLE 1.—*Backcross and F_2 data for the factor S' for self-fertility*

Hybrid	Population No.	Self-fertile plants	Self-sterile plants
Self-sterile × heterozygous self-fertile, $S^aS^b \times S'S^x$:		*Number*	*Number*
08-1 × 01a-1 [1]	[2] 1166	[2] 49	[2] 44
Clone 91 × 2222-150	4382	12	13
Clone 113 × 2222-150	4480	6	4
Clone 139 × 4356A-668	5212	11	10
Clone 139 × 4356A-671	5213	34	37
Clone 139 × 4356A-673	5214	27	30
Clone 139 × 4712-676	5237	7	7
Clone 137 × 5170	6159	38	29
5563-1 and 3 × 5214	6283	22	13
Total		206	187
Heterozygous self-fertile, $S'S^a$ (or $S'S^b$) selfed:			
4356A-1 selfed	5369	7	0
4356A-668 selfed	5373	40	0
4356A-673 selfed	5375	17	0
4514-635 selfed	5426	23	0
4514-643 selfed	5428	10	0
Total		97	0
Self-sterile × homozygous self-fertile, $S^aS^b \times S'S'$.			
Clone 4 × 2255-206	3139	108	2
Clone 90 × 2255-7	4356A	7	0
Clone 97 × 2255-7	4407	15	0
3474-35 × 2255-7	4712	16	0
Total		146	2

[1] The population obtained by the self-fertilization of 01a-1 became known as 1167.
[2] Data from population 1166 supplied by F. A. Abegg, Division of Sugar Plant Investigations, Bureau of Plant Industry.

The combined results of crosses between self-steriles $S^a S^b$ and heterozygous self-fertiles $S'S^x$ used as pollen parents (table 1) show 206 self-fertile and 187 self-sterile plants, which is a fairly good 1 : 1 ratio. This 1 : 1 ratio is in accord with the hypothesis of a single factor for self-fertility.

Selfing heterozygous self-fertile plants $S'S^a$ (or $S'S^b$) produced a total of 97 self-fertile plants (table 1) but no self-sterile plants. This indicates that pollen with allelomorphs S^a or S^b was unable to produce self-fertilization on $S'S^a$ (or $S'S^b$) plants.

Homozygous $S'S'$ plants were demonstrated in population 2255. Four populations resulting from self-sterile seed parents $S^a S^b \times$ homozygous 2255, $S'S'$, plants are shown in table 1. All the offspring were expected to be self-fertile, and from 148 plants tested 146 were observed to be self-fertile and 2 self-sterile. The 2 self-steriles may have come from the scant self-fertilization of the self-sterile seed parents, which were not emasculated in making the crosses.

TABLE 2.—*Backcross data for S' and R*

Population No	$R\ S'$ plants	$R\ S^x$ plants	$r\ S'$ plants	$r\ S^x$ plants
	Number	*Number*	*Number*	*Number*
588	8	8	10	10
590	4	7	6	7
5212	4	7	4	6
5213	17	17	26	24
5214	12	15	15	15
6159	21	17	14	15
6283	14	8	6	7
Total	80	79	81	84
Expected with R and S' independent	81	81	81	81

TABLE 3.— *F_2 data for S' and R from $R\ r\ S'S^x$ plants selfed*

Population No.	Individuals			[1] χ^2
	R	r	Total	
	Number	*Number*	*Number*	
8510	746	259	1,005	0 319
8512	763	272	1,035	.905
8514	260	58	318	7.753
8519	286	95	381	.001
9538	88	24	112	.762
Total	2,143	708	2,851	.042

[1] For each χ^2 there is 1 degree of freedom; $\chi^2 = 6.635$ indicates a discrepancy equal to the 1-percent level of significance (*14*).

Independent inheritance between self-fertility produced by S' and the red hypocotyl factor R (*16*) is indicated in backcross populations (table 2). The observed distribution was 80 : 79 : 81 : 84, where the theoretically expected number, assuming independence between S' and R, is 81 plants for each class. Independent inheritance between S' and R is also shown by F_2 data (table 3). The F_2 populations were secured by selfing $R\ r\ S'S^x$ plants, where, if linkage is assumed, R and S' entered in the coupling phase. In selfing heterozygous plants, $S'S^x$, it has been shown (table 1) that, in general, only S' pollen

F₁ HYBRID

GENOTYPES S^1 S^2 Z^3 Z^4 X S^F S^5 Z^6 Z^7

FUNCTIONAL GAMETES
1-3 1-4 2-3 2-4 F-6 F-7 5-6 5-7

SELF-FERTILE   SELF-STERILE

1st BACKCROSS

GENOTYPES S^1 S^2 Z^3 Z^4 X S^F S^1 Z^3 Z^6

FUNCTIONAL GAMETES
1-3 1-4 2-3 2-4 F-3 F-6 1-6

SELF-FERTILE   SELF-STERILE

2D BACKCROSS CLASS A

GENOTYPES S^1 S^2 Z^3 Z^4 X S^F S^1 Z^3 Z^4

FUNCTIONAL GAMETES
1-3 1-4 2-3 2-4 F-3 F-4

ALL SELF-FERTILE

2D BACKCROSS CLASS B

GENOTYPES S^1 S^2 Z^3 Z^4 X S^F S^1 Z^3 Z^6

FUNCTIONAL GAMETES
1-3 1-4 2-3 2-4 F-3 F-6 1-6

SELF-FERTILE   SELF-STERILE
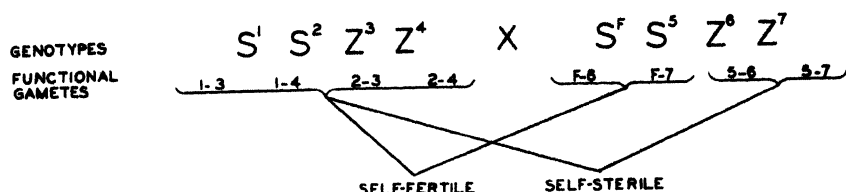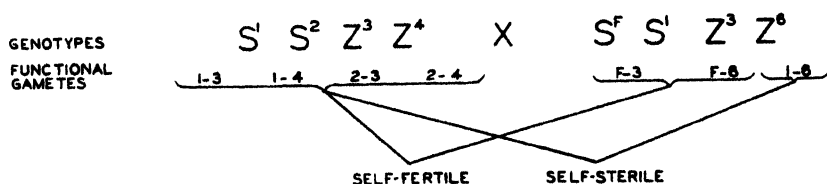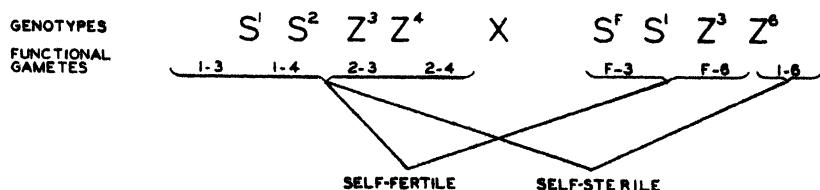
FIGURE 5.—Results of self-fertility expected in the F_1 generation and in back-crosses, with duplicate factors responsible for cross-sterility. With parental constitutions as indicated, a 1 : 1 ratio of self-fertile to self-sterile plants is expected in the F_1 generation, and a 2 : 1 ratio of self-fertile to self-sterile plants is expected in the first backcross. There are two types of populations in the second backcross generation: Class A, giving all self-fertiles; and class B, giving 2 : 1 ratios of self-fertile to self-sterile plants. Gametes are indicated by showing superscripts only, 1–3 representing gametes S^1Z^3, etc.

functions. Therefore, if R were completely linked with S' all F_2 plants should have carried R and any significant amount of linkage would be revealed by a deficiency of r plants. In table 3, a deficiency of r plants is indicated for population 8514 (260 R:58 r plants), but the other 4 F_2 populations show normal 3:1 ratios. The combined results for the 5 populations show 2,143 R to 708 r plants, which is a good 3:1 ratio and indicates independence between S' and R.

The presence of duplicate sterility factors is indicated by the behavior of successive backcrosses to the self-sterile clone 144 (table 4 and fig. 5). Clone 144, arbitrarily designated $S^1S^2Z^3Z^4$, was crossed with the self-fertile clone 70. Clone 70 proved to be heterozygous for self-fertility and, to illustrate theoretical assumptions, is assumed to be of constitution $S'S^5Z^6Z^7$. Segregation was observed in the F_1 population with 4 self-fertile plants and 2 self-sterile plants (table 4). In the first backcross generation there were 11 self-fertile and 5 self-sterile plants. In the second backcross generation there were 52 self-fertile but no self-sterile plants. These results are all in agreement with expectation based on the assumption of duplicate sterility factors.

TABLE 4.— *Inheritance of self-fertility in successive backcrosses to self-sterile clone 144*

Hybrid or backcross	Population No.	Self-fertile plants	Self-sterile plants
		Number	*Number*
F_1 generation, $S^1S^2Z^3Z^4 \times S'S^5Z^6Z^7$			
Clone 144×clone 70	4514	4	2
First backcross, $S^1S^2Z^1Z^4 \times S'S^1Z^3Z^6$ [1]			
Clone 144×4514 635	5247	11	5
Second backcross, $S^1S^2Z^3Z^4 \times S'S^1Z^3Z^4$ [2]			
Clone 144×5247 3	6169	52	0

[1] $S'S^1Z^3Z^6$ is 1 of 8 different self-fertile genotypes expected in the F_1, all of which, when used as pollen parents, produce 2 : 1 ratios of self-fertile to self-sterile offspring in the first backcross generation

[2] $S'S^1Z^4Z^4$ is 1 of 4 different self-fertile genotypes expected in the first backcross which, when used as pollen parents, produce nothing but self-fertile offspring in the second backcross. Four other genotypes, $S'S^1Z^3Z^6$, etc., if used similarly, produce 2 : 1 ratios of self-fertile to self-sterile plants, as in the first backcross generation. (See fig. 5)

The nature of expected results in successive backcrosses to a self-sterile plant is illustrated in figure 5. For convenience the self-sterile plant is assumed to be of constitution $S^1S^2Z^3Z^4$ and the original self-fertile pollen plant, $S'S^5Z^6Z^7$. With a pollen parent of the type $S'S^5Z^6Z^7$, heterozygous for S', a 1 : 1 ratio of self-fertile to self-sterile plants is expected in the F_1 generation. If the pollen parent were homozygous for S' all F_1 plants would be self-fertile, but the type of results expected in backcrosses would remain the same.

Of the possible 16 different F_1 genotypes that may be derived from the parental gametes indicated in figure 5, eight ($S'S^1Z^3Z^6$, $S'S^1Z^4Z^6$, etc.) carry S' and should be self-fertile. The behavior, with regard to segregation for self-fertility in successive backcrosses, is the same irrespective of which of the eight self-fertile genotypes is used as a pollen parent in the first backcross. Self-fertile F_1 plants produce four types of gametes. The F_1 $S'S^1Z^3Z^6$, for instance, may produce $S'Z^1$, $S'Z^6$, S^1Z^3, and $S'Z^6$ gametes, but S^1Z^3 pollen is expected to be sterile in a backcross to the maternal parent $S^1S^2Z^3Z^4$. The sterility of the S^1Z^3 class of pollen accounts for the 2 : 1 ratio of self-

fertile to self-sterile plants in the first backcross generation. In the second backcross generation two classes are expected, class A and class B (fig. 5). The theory requires that in class A all the offspring should be self-fertile and in class B there should be a 2:1 ratio for self-fertile to self-sterile plants. Class A and class B populations are expected with equal frequency.

Class A populations in the second backcross generation involve self-fertile pollen plants of the type $S'S^1Z^3Z^4$ with three of the four genes in common with the self-sterile maternal parent $S^1S^2Z^3Z^4$. From $S'S^1Z^3Z^4$ plants pollen carrying S' is the only pollen that is functional with the $S^1S^2Z^3Z^4$ clone; hence, all the offspring carry S' and are expected to be self-fertile. In table 4 it appears that the 52 self-fertile plants in the second backcross generation, population 6169, may be accounted for by the fact that it was of the class A type. The class B type of population, resembling the first backcross, is not represented in backcrosses to clone 144 (table 4).

Difficulty is encountered in attempting to explain backcrosses to clone 90 on the basis of duplicate factors for sterility. The results for these backcrosses are shown in table 5. Clone 90, rrS^aS^b, was first crossed with the self-fertile plant 2255–7, $RRS'S'$, and the resulting F_1 population was labeled 4356A. Clone 90 was then backcrossed through three generations to its own red self-fertile offspring ($RrS'S^a$ or $RrS'S^b$).

TABLE 5.—*Inheritance of self-fertility in successive backcrosses to self-sterile clone 90*

Hybrid or backcross	Population No.	Self-fertile plants	Self-sterile plants
F_1 generation, $S^aS^b \times S'S'$. [1]		*Number*	*Number*
Clone 90×2255–7	4356A	7	0
First backcross populations, $S^aS^b \times S'S^a$ (or $S'S^b$):			
Clone 90×4356A–671	588	16	20
Clone 90×4356A–673	590	11	13
Total		27	33
Second backcross populations, $S^aS^b \times S'S^a$ (or $S'S^b$):			
Clone 90×590–17	6118	57	[2] 27
Clone 90×590–19	6119	13	12
Third backcross populations, $S^aS^b \times S'S^a$ (or $S'S^b$):			
Clone 90×6118	7128	23	[2] 5

[1] Evidence for a duplicate set of Z factors (table 4) considered in text, but, since all results with clone 90 backcrosses cannot be explained by duplicate factors, only the S series of factors is indicated here.

[2] Deficiency of self-sterile plants, as compared with the number of self-fertile plants, in populations 6118 and 7128, indicates selective action against S^a or S^b pollen.

In the first backcross generation a total of 60 plants was tested (populations 588 and 590, table 5) of which 27 were self-fertile and 33 were self-sterile, which is a good 1:1 ratio. This 1:1 ratio is not in agreement with expected results based upon the theory of duplicate sterility factors. A 2:1 ratio of self-fertile to self-sterile plants is expected with duplicate factors for sterility (fig. 5). Since a total of 60 plants were tested and 33 were observed to be self-sterile, there is a deviation of 13 from expectation, which is over 3 times the standard error of 3.65.

Results with population 6118, which represents a second backcross generation, show 57 self-fertile plants to 27 self-sterile plants, which is a good 2:1 ratio and agrees with results expected with duplicate for cross-sterility (fig. 5). Population 6119, however, which

is also a second generation backcross, produced 13 self-fertile to 12 self-sterile plants; hence, these results agree more closely with a 1:1 ratio.

Population 7128 represents a third backcross generation. Of 28 plants tested, 23 were self-fertile and 5 were self-sterile (table 5). The deficiency of self-sterile plants is too great to suggest a 2:1 ratio of self-fertile to self-sterile plants.

Since the backcross data with clone 90 do not clearly fall in line with the theory of duplicate factors, it is possible that some other auxiliary factors may be involved. All backcross data, however, both with clone 144 (table 4) and clone 90 (table 5), show that a single series of S factors cannot control cross-sterility. If a single series of S factors determined cross-sterility, all plants in the first backcrosses should have been self-fertile, because the maternal plants S^aS^b would have been sterile to the S^a (or S^b) pollen from F_1 $S'S^a$ (or $S'S^b$) plants. For S^a (or S^b) pollen to be effective in backcrosses to self-sterile parental clones, it is necessary to assume the presence of additional factors. The second and the third backcross generations represent a higher degree of inbreeding with a likelihood of greater similarity of genotypes between plants used for pollen parents and maternal parents. This closer approach to the genotype of the maternal parents may have been responsible for greater dependence upon the one allelic series of factors under investigation, with a greater deficiency of self-sterile plants in second and third backcross populations as indicated in tables 4 and 5.

Special attention was given to the 27 self-sterile plants in population 6118, a second backcross to clone 90 (table 5). Two of these 27 self-sterile plants produced aborted pollen. Nine of the self-sterile plants with normal pollen were subjected to further study. Clone 90, S^aS^b, was fully fertile to the pollen from seven of these nine plants of population 6118, assumed to be S^aS^a and S^aS^b (or S^bS^b and S^aS^b). Results with the seventh plant, G (table 6), were negative but questioned, and the eighth plant was not tested. Six of the self-sterile plants, A, B, D, G, H, and I (table 6), were fully fertile to pollen of clone 90, and three plants, C, E, and F, were rather poorly fertile. Thirteen pollinations between respective self-sterile plants of population 6118 were also attempted. Seven of these crosses were fertile, and combinations E×H and H×C were the only two definitely incompatible combinations discovered. None of the self-sterile genotypes in population 6118 (S^aS^a and S^aS^b or S^bS^b and S^aS^b) could be fertile in either direction with clone 90 (S^aS^b) if S^a and S^b alone determined cross-sterility. Apparently the presence of duplicate or auxiliary factors made it possible to secure plants homozygous for S^a and S^b.

TABLE 6.—*Compatibility of self-sterile segregates from the second backcross population 6118*

Clone 90 as parent	Compatibility [1] of self-sterile plants A to I in population 6118 with clone 90								
	A	B	C	D	E	F	G	H	I
Clone 90 as female	+	+	+	+	+	+	(2)	(2)	+
Clone 90 as pollen parent	+	+	P	+	P	P	+	+	+

[1] Designations: +=fertile; P=poorly fertile.　[2] Results negative but questioned.　[3] Not tested.

SELF-FERTILITY BY MUTATION FROM THE 12C STRAIN

The self-sterile clone 4, of the 12c strain, previously described and used extensively in connection with studies of cross-sterility, gave rise to a rather remarkable instance of self-fertility. Presumably the factor S^f for self-fertility arose from S^x by mutation.

FIGURE 6.—Beets under heavy curly top exposure with uniform soil fertility and cultural care. Photographed September 21, 1940, at Salt Lake City, Utah. *A*, Strain 1167, used extensively for source of self-fertility factor S^f; *B*, an S_2 population from clone 4 of the 12c strain, with self-fertility apparently derived by mutation; *C*, susceptible European variety R. & G. Old Type.

Four vigorous vegetative cuttings from clone 4 were planted in a city-garden isolation in Ogden, Utah, in 1933. These plants grew well and flowered normally, but very few of the flowers were fertilized and only a scant supply of poorly developed seed was produced. This small supply of seed was carefully planted in a greenhouse.

Twenty seedlings grew from this seed, but all were either red garden beet or Swiss chard hybrids, except four plants that resembled the parental plant, clone 4, and looked like sugar beets. The male parentage of these four plants was of course much in doubt. Since pollen from distant and unknown sources of red garden beets and Swiss chard reached the isolation, the four seedlings resembling sugar beets may also have arisen from hybridization with distant sources of sugar-beet pollen. Without knowing their male parentage, the four plants were grown to the flowering stage and their sterility reactions were studied. One of these plants was very exceptional because of a high degree of self-fertility.

Self-fertilized S_2 seed produced under paper bags was collected from this self-fertile plant. From this S_2 seed, 29 plants were grown and tested, and 28 were found to be highly self-fertile.

If this self-fertility arose from self-fertilization of clone 4, then mutation appears to be the only logical explanation. Certain facts indicate that the original self-fertile plant probably did arise by mutation rather than by contamination from a distant source of pollen. The curly top resistance and uniformity in bolting tendency of the offspring were factors furnishing conclusive evidence. Clone 4 was resistant to curly top but not so highly resistant as strain 1167. In 1933, when clone 4 plants were isolated at Ogden, curly top resistant varieties were not available to commercial growers; therefore, any sugar-beet pollen likely to have reached the isolation would have been from European varieties susceptible to curly top.

The S_2 population derived from selfing the original self-fertile plant showed a high degree of uniformity for a fast bolting tendency similar to that of clone 4. Twenty S_3 populations derived from selfing the S_2 plants were tested for curly top resistance in 1940. The degree of uniformity in leaf type and in curly top resistance was sufficient to rule out any possibility of origin through hybridization in the 1933 isolation with a susceptible variety. The S_3 plants in the 20 S_3 populations, when grown under a heavy curly top exposure, were remarkably uniform for the rather unusual leaf type and intermediate degree of curly top resistance shown in figure 6, *B*.

DISCUSSION

Any hypothesis to account for the observed results on self- and cross-sterility in sugar beets must involve some complexity. It was found necessary to assume the presence of duplicate oppositional factors to give a satisfactory explanation of the cross-sterility data. Unknown auxiliary factors apparently further complicated some of the results on cross-sterility. In contrast with the complexity of inheritance of cross-sterility, the inheritance of a high degree of self-fertility in sugar beets was found to be produced by the single factor S'.

The simplicity of the inheritance of self-fertility is of much interest to the plant breeder. The knowledge that a single factor, S', produces self-fertility simplifies the problem of obtaining inbred lines. With only a single factor involved, it becomes a simple matter to transfer the self-fertility character to any material desired. As soon as the factor S' is introduced, inbred lines can be easily produced under paper bags or by any other feasible means of isolation. This factor for self-fertility has been added to a wide variety of curly top resistant

strains of sugar beets at the Salt Lake City laboratory of the Division of Sugar Plant Investigations.

The F_1 hybrids between self-sterile and self-fertile plants, heterozygous for the factor S', have shown a remarkably high degree of self-fertility. This increased self-fertility seems to be associated with hybrid vigor. Large quantities of pollen are produced by these vigorous F_1 plants. In several instances the degree of self-fertility apparently decreased with inbreeding. Sundelin (*30*) reported similar observations and made what appears to be a logical assumption that the apparent decrease in degree of self-fertility sometimes associated with continued inbreeding is caused by a general decrease in vigor. The inbred lines that lack vigor have been inclined to be slow to flower, thus encountering hotter and more unfavorable weather under field conditions. In some instances inbred lines have been very poor pollen producers.

Full utilization of self-fertility in breeding work, however, is dependent upon further information, because there are certain disadvantages in self-fertility when hybridization is desired. Recovery of self-sterility from self-fertile plants is not difficult as long as the factor S' for self-fertility is held in the heterozygous condition. Hybrids between self-steriles $S^a S^b$ and heterozygous self-fertiles $S'S^x$ yielded 50 percent self-steriles $S^a S^x$ (or $S^b S^x$), and self-sterile segregates were easily identified by means of tests with paper bags. However, the maintenance of the factor S' in the heterozygous condition requires continuous backcrossing to self-sterile plants, which may not be a practical procedure unless some simplified methods can be devised. Knowledge of linkage between the factor S' and other Mendelian characters would be of particular interest. No linkage between the factor S' and the factor R for red hypocotyl and plant color is indicated in data reported (tables 2 and 3).

The existence of intermediate or partly self-fertile types, which were largely avoided in the present studies, adds further complexity to genetic interpretations. Indications of embryo abortion associated with this partly self-fertile condition were observed in the Tracy strain 2769. These plants were not self-fertile, nor could they be considered as typically self-sterile. The pericarps of the seed balls under paper bags in the greenhouse swelled quickly after the flowers opened, a behavior similar to that of fully self-fertile plants, and the stigma lobes disintegrated quickly, making the use of paper bags impossible in the work of hybridization. Nevertheless, these plants produced nothing but empty seed balls, while known self-fertile plants under the same conditions produced good viable seed. From the limited observations made, it appeared that fertilization had probably been completed in many cases and that death of the embryo was the cause of these empty seed balls. These observations are in agreement with those of Kharechko-Savitskafa (*17, 19*), who has described death of the beet embryo as a common result of self-fertilization. Brink and Cooper's (*5*) work on alfalfa indicates that death of the young embryo may commonly be associated with self-fertilization.

SUMMARY

The degree of self-sterility in *Beta vulgaris* L. was found to be determined by both environmental and heritable influences. Under greenhouse conditions end-season self-fertility was pronounced with

certain self-sterile plants. Intermediate types with a partly self-fertile condition, which was clearly heritable, were observed, but these types were avoided in attempting a genetic analysis of self-sterility and fertility.

Cross-sterility was found to be common in the curly top resistant line 12c. This cross-sterility was frequently nonreciprocal in behavior. An explanation based on a duplicate series of oppositional factors explains most of the results.

A highly self-fertile condition was produced by a single Mendelian factor designated S^f. The expression of the factor S^f was clearly defined; but identification of other S factors, believed associated with self-sterility and allelic to S^f, was difficult owing to the presence of unknown auxiliary factors or to duplicate factors that were not identified. Self-sterile plants were designated S^aS^b; and F_1 hybrids with self-fertiles, S^fS^a or S^fS^b. The S^a (or S^b) pollen did not produce self-fertilization on F_1 S^fS^a plants. The heterozygous S^fS^a plants bred true for self-fertility, presumably with the production of only S^fS^f and S^fS^a offspring. No linkage was detected between the factor S^f for self-fertility and the factor R for red plant and hypocotyl color. One instance is reported where self-fertility arose suddenly in a self-sterile line, presumably by mutation.

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GENETICS OF PSEUDO-SELF-COMPATIBILITY AND ITS RELATION TO CROSS-INCOMPATIBILITY IN TRIFOLIUM REPENS[1]

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INTRODUCTION

When a large number of white clover (*Trifolium repens* L.) plants, grown from open-pollinated seed, were self-pollinated under bag in the field (*3*),[2] a few were self-incompatible, a few others were as self-compatible as they were cross-compatible, but the large majority were intermediate, setting only a few seeds per head. This intermediate self-compatibility has been called "pseudo-self-compatibility" by previous workers, and the term will be used here in that general sense. Much variation has been found regularly in pseudo-self-compatibility, not only between plants but also within plants. No method has been found to eliminate the latter variation, but all mean values have been based on at least several heads. Williams (*8*) has described plants of white clover that set only a few seeds per head, but he did not report on the inheritance of this character. Since this ability of a plant to set selfed seeds, even though relatively low in self-compatibility, has obvious importance in a breeding program, it was desired to determine the genetic mechanism responsible and its relation to that causing self- and cross-incompatibility (*1*).

MATERIALS AND METHODS

The female parent of the *Trifolium repens* used in this investigation came from seed collected in Maryland, and the male parent from seed collected in New York. The cross between them was made in the winter of 1937–38, with clonal increases grown in the greenhouse. The F_1 population was planted in the field in 1938, the F_2 in 1939, and the F_3 in 1940. Every plant of the progeny has been similar to the two parents in having solid green leaves, the recessive condition to most of the common leaf markings found. The plants selected for greenhouse study were chosen principally for their vigor, good flowering, and freedom from disease. All crosses were made in screened greenhouses during the winter. The techniques used for emasculating and pollinating in the greenhouse, as well as for self-pollination under bag in the field, have already been described (*3*).

EXPERIMENTAL RESULTS

BEHAVIOR OF PARENT PLANTS

During their first summer in the field (1937) about one-half of the 10,000 plants in the nursery flowered. Over 200 of these were bagged, and the plant setting the highest number of seeds was chosen as the

TABLE 1.—*Number of seeds set per 10 flowers in all possible reciprocal combinations between 13 pseudo-self-compatible sister plants (2–3 to 2–15) resulting from the previous cross between a pseudo-self-compatible female parent (2–1) and a self-incompatible male parent (2–2), together with the reciprocal backcrosses of each F_1 plant with both parents and the seed set by each of these plants under bag in the field*

Female parent			Seeds set [1] after pollination with indicated plant of group—																						Aver-age for com-patible crosses	Selfed seeds per head in field in—	
Group No.	Geno-type	Plant No.	2–I (S_4S_5) 2–1	2–II (S_6S_7) 2–2	2–III (S_4S_5) 2–9		2–12	2–3	2–IV (S_6S_8) 2–7	2–11	2–14	2–4	2–6	2–V (S_4S_7) 2–10	2–13	2–5	2–8	2–VI (S_6S_7) 2–15									1939	1940
2–I	S_4S_5	2–1	0	48	43	43	43	47	44	41	50	45	45	46	36	46	45	45	44.6	*No.* 51.8	*No.* 37.5
2–II	S_6S_7	2–2	53		53		56	55	52	55	54	50	57	52	[57]	52	56	52	53.7	2.8	.9
2–III	S_4S_8	{ 2–9	0	41	37			24	39	42	41	47	(45)	42	38	42	38	46	40.4	1.9	3.0
		2–12	0	46	38	0		46	45	40	48	40	(41)	49	47	49	47	49	45.3	5.0	10.4
2–III		{ 3 2–9	0	29	31	0	26	0	0	0	42	45	28	22	38	(36)	39	36	40.1	21.0	13.2
2–IV	S_5S_8	{ 2–3	0	32	33	1	42	4	0	0	1	35	50	45	42	44	46	38	32.8	6.7	48.4
		2–7	35	44			37				2	36	34	44	31	30	36	41	41.9	24.7	4.1
		2–11	41	36			32				2	36	(27)	39	32	32	36	32	36.0		
		3 2–14	33																33.1		
2–IV		34																			
2–V	S_4S_7	{ 2–4	0	(40)	48	48	37	53	48	51	38	0	0	42	0	49	48	39	45.5	11.1	3.1
		3 2–6	0	46	(37)	38	42	42	37	42	46	(37)	0	38	0	38	35	48	41.0	.9	7.6
		2–10	1	34	41	42	37	44	40	50	43	52	0	45	0	45	36	42	41.8		8.3
		2–13	0	49	44	38	32	44	49	44	42		1	(49)	0	(49)	47	45	45.1		
2–V																					
2–VI	S_5S_7	{ 2–5	46	42	39	41	42	45	40	40	48	38	37	41	43	0	0	0	41.7	13.0	19.3
		3 2–8	37	34	30	40	43	31	39	(39)	44	(37)	38	31	38	1	2	0	36.5	5.0	
		2–15	46	43	45	47	46	46	50	48	47	52	49	40	47	0	0	1	46.7		
Average of F_1																				40.8	9.8	13.1

[1] Values enclosed in heavy black lines are incompatible crosses or selfs.
[2] Numbers in parentheses in this and the following tables have been adjusted to a 10-flower basis. For instance, if a pod was lost at harvesting, the total number of seeds obtained was increased by ⅒. In most cases at least 8 flowers were available.
[3] Plants selected in each group as parents of the F_2 population.
[4] Duplicate cross yielded no seed.

female parent (plant 2–1 or group 2–I in all tables).   The 2 heads enclosed at that time averaged 115 seeds per head.   In the following winter in the greenhouse, the manipulated heads averaged 55.4 seeds, while the next summer in the field only 21.5 seeds per head were obtained.   As shown in table 1, the averages for 1939 and 1940 were 51.8 and 37.5, respectively.   With bee pollination under a cage (*3*), this plant averaged only 19.4 seeds per head.   Although the inter-annual variation was very great, this parent appeared to represent an extreme type of pseudo-self-compatibility.   Few other plants have averaged as many seeds per head without also giving evidence of true self-compatibility (*2*).

It should be noted that pseudo-self-compatibility is brought into expression only by manipulation of the heads.   In the greenhouse during 1938–39, 10 flowers on 1 head of the female parent were selfed following emasculation, 2 similar heads were selfed without emascula-tion, and 1 was selfed in the bud.   One of the unemasculated heads bore 4 seeds, but the others had none.   Similar results were obtained with the F_1 and F_2 plants.   When an abundance of pollen was placed on the stigma by hand in the greenhouse, these plants proved almost self-incompatible.   Yet with the supposedly much less precise pollina-tion resulting from rubbing of entire heads, the same plants showed a high degree of pseudo-self-compatibility.   A similar behavior has been found with other unrelated pseudo-self-compatible plants.   When a large amount of self pollen is placed on the stigma, the incompatibility reaction is so potent as to prevent almost all pollen tubes from growing through the style (*4*).   On the other hand, it has been observed that with manipulation relatively few pollen grains adhere to the stigma.   In this case, the incompatibility reaction may not be so strong, and there may be a better chance for fertilization.

The original male parent (plant 2–2 or group 2–II in all tables) yielded no seed when manipulated under bag in 1937.   The following winter in the greenhouse it produced by manipulation only 1.5 seeds per head, but the next summer in the field it again produced no selfed seed.   Similarly, in 1939 and 1940 its seed set was very low (table 1); comparatively speaking, this plant may be considered practically self-incompatible.

GENETICS OF CROSS-INCOMPATIBILITY AMONG PSEUDO-SELF-COMPATIBLE PLANTS

The seed set obtained when crossing reciprocally in all combinations the 13 selected F_1 plants and also the seed set from the reciprocal back-crosses to both parents are shown in table 1.   Almost every cross was made twice and a few were made three times, the replicates usually being made by different persons, some on the same day and others at various intervals up to 7 weeks.   Since the largest number of seeds obtained was considered the most accurate measure of the true poten-tialities of a combination, only the higher or highest seed set of the replicates is recorded in table 1.   Lower numbers probably resulted from poor pollination or from damage during crossing and seed ripening rather than from reduced compatibility.   Although replicates usually varied somewhat in number of seeds, it was generally not difficult to decide whether a particular head had been involved in a compatible or an incompatible mating, and in no case did replicates give different compatibility results.   The average of the higher seed set among the compatible F_1 intercrosses was 40.8 per 10 flowers, whereas the average

obtained from 104 incompatible F_1 crosses and selfs, including repli-
cates, was 0.18. About one-half of these seeds produced in the green-
house from incompatible matings proved contaminants when a sample
of them was tested for possible homozygosity. In other words, the
average of the incompatible matings should really be about 0.09,
which effectively doubles the ratio between compatible and incompat-
ible crosses in average seed set.

Four intrasterile, interfertile groups of 4, 4, 3, and 2 plants, respec-
tively, were found among the F_1 population. From this it can be
inferred that cross-incompatibility among pseudo-self-compatible
plants is determined by a series of multiple oppositional factors (6),
and that the heterozygous parents used here differed in all four factors
which they carried. According to this hypothesis, all backcrosses to
both parents should be compatible in both directions. As shown in
table 1, however, the backcrosses of two of the F_1 groups (2-III and
2-V) with the original female parent (2-I) as male were incompatible.
This difference between reciprocal backcrosses can be explained best
on the assumption that the plant involved (2-I) is homozygous for
S factors; but, as already pointed out, it must originally have been
heterozygous in order to yield four F_1 groups when crossed with 2-II.
It is postulated, therefore, that subsequent to the original cross either
a mutation occurred to make this plant homozygous or a contamina-
tion occurred in the field. The plant was perpetuated in the field as
clonal slips between the time of the original cross and the time of the
backcrosses with the F_1 plants. Since all other results with this plant
have been interpreted best on the assumption that all supposed clones
of the female parent were homozygous, the plant is so designated in
tables 4 and 5.

Because the genetic explanation of cross-incompatibility among
pseudo-self-compatible plants is similar to that for self-incompatible
plants, it seemed desirable to determine whether any of the S factors
in the plants used here were identical with those previously reported
(1). The four homozygous genotypes obtained in the 2-series (see
p. 704) were crossed as males (table 2) onto the six groups of the 1-series

TABLE 2.—*Number of seeds set per 10 flowers when the 4 homozygous plants of the
2-series were crossed onto the 6 groups of the 1-series*

Female parent		Seeds set [1] after pollination with plant of genotype—				
Group No.	Genotype	S_4S_4	S_5S_5	S_6S_6	S_7S_7	Average for compatible crosses
		Number	*Number*	*Number*	*Number*	*Number*
1-I	S_1S_2	52	51	47	43	48.2
1-II	S_3S_4	0	[2] 15	[2] 18	31	31.0
1-III	S_1S_1	38	40	37	38	38.2
1-IV	S_2S_3	52	51	56	51	52.5
1-V	S_1S_4	0	46	53	47	48.7
1-VI	S_2S_4	1	46		[2] 24	46.0

[1] Values enclosed in heavy black lines are incompatible crosses.
[2] Cross was recorded as compatible by reflection of pedicels on third day after pollination. This abnor-
mally low seed set was omitted from average for plant.

described in a previous paper (1). The homozygous genotype that
was incompatible with one parental (1-II) and two F_1 groups (1-V

and 1–VI) must have borne the gene common to these three groups, namely S_4. Since all other combinations were compatible, the other three factors in the 2-series must have been different from those in the 1-series, and they have been called S_5, S_6, and S_7. A second test confirmed these results. When four intrasterile, interfertile groups are found in an F_1 population, these four groups, together with those of the two parents, constitute the six possible combinations in pairs of the four genes brought together in the original cross. The second test, therefore, consisted of crossing in all combinations the six groups found in the 1-series (*1*) with those found in the 2-series. With the groups of the 2-series as female, all 36 combinations were compatible, but with the 1-series as female, three of the 36 combinations failed. These involved the matings of 2–I as male with one parental (1–II) and two F_1 groups (1–V and 1-VI) of the 1-series. Here again, reciprocal matings are different, and they provide evidence not only that one gene of the 2-series must be the same as one in the 1-series, but that the particular gene is homozygous in the parent plant 2–I.

In order to assign genotypes to the six groups and to test further the hypotheses used to explain the reaction of 2–I, an F_2 test was carried out similar to that with the 1-series (*1*). Three F_1 intercrosses, involving matings of one of the F_1 groups as female with each of the other three, were selected for further test. In the case of the 1-series, a gene from each parent was arbitrarily assigned to one of the F_1 groups, and the other three groups were defined by their reaction with this first group. Here, however, one of the genes, S_4, is already defined because of its previous designation in the 1-series. Since the group used as female in these crosses did not bear S_4 (see reaction of 2–I in table 1), this group must have borne the other gene from the original female, together with one from its male parent. The genes assigned to this group were S_5 and S_6, and it has been called group 2–IV. The genotypes of the 2-series are as follows:

$$\overset{\text{(I)}}{S_4 S_5} \times \overset{\text{(II)}}{S_6 S_7}$$

$$\overset{\text{(III)}}{S_4 S_6} + \overset{\text{(IV)}}{S_5 S_6} \downarrow \overset{\text{(V)}}{S_4 S_7} + \overset{\text{(VI)}}{S_5 S_7}$$

The three F_1 intercrosses should then yield as follows:

$$\overset{\text{(IV)}}{S_5 S_6} \times \overset{\text{(III)}}{S_4 S_6} \longrightarrow \overset{\text{(I)}}{S_4 S_5} + \overset{\text{(III)}}{S_4 S_6}$$

$$\overset{\text{(IV)}}{S_5 S_6} \times \overset{\text{(V)}}{S_4 S_7} \longrightarrow \overset{\text{(I)}}{S_4 S_5} + \overset{\text{(III)}}{S_4 S_6} + \overset{\text{(VI)}}{S_5 S_7} + \overset{\text{(II)}}{S_6 S_7}$$

$$\overset{\text{(IV)}}{S_5 S_6} \times \overset{\text{(VI)}}{S_5 S_7} \longrightarrow \overset{\text{(VI)}}{S_5 S_7} + \overset{\text{(II)}}{S_6 S_7}$$

When 38 F_2 plants were tested by backcrossing to the P and F_1 groups, only expected groups were obtained (table 3). This confirms the diploid personate theory of multiple oppositional factors as an explanation of cross-incompatibility among pseudo-self-compatible plants of white clover. Although the segregating progenies were small, the agreement between expected and obtained ratios was satisfactory

TABLE 3.—*Segregations of intrasterile, interfertile groups in F_1 and F_2*

Cross	Generation	2-I Expected	2-I Obtained	2-II Expected	2-II Obtained	2-III Expected	2-III Obtained	2-IV Expected	2-IV Obtained	2-V Expected	2-V Obtained	2-VI Expected	2-VI Obtained	Total plants Expected	Total plants Obtained
		No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
2-I×2-II	F_1	-----	-----	-----	-----	3.25	2	3.25	4	3.25	4	3.25	3	13	13
2-IV×2-III	F_2	5	5	-----	-----	5	5	-----	--	-----	--	-----	-----	10	10
2-IV×2-V	F_2	4.5	4	4.5	5	4.5	4	-----	-	-----	--	4.5	5	18	18
2-IV×2-VI	F_2	--	-----	5	6	-----	---	-----	-----	-----	--	5	4	10	10

in every case. Not all possible reciprocal combinations between F_2 plants and the 6 test groups were made, but there were enough crosses with every plant to be certain of its genotype. The reflection of the pedicels (*1*) was a good measure of compatibility with these plants, so that few of the F_2 crosses were made in duplicate. Here, as with the F_1 plants, no difficulty was generally experienced in distinguishing compatible from incompatible crosses. Since the data are similar to those obtained with self-incompatible plants (*1*), seed set is shown from only 4 F_2 plants (table 4). The types of behavior shown_here are characteristic of those obtained with all 38 F_2 plants.

TABLE 4.—*Number of seeds set per 10 flowers when typical F_2 plants from cross 2-IV×2-V (2-14×2-6) were backcrossed to P and F_1 groups*

Group No.	Genotype	Plant No.	As female or male parent	Seeds set [1] after backcrossing to group 2-I (S_4S_4)	2-II (S_6S_7)	2-III (S_4S_6)	2-IV (S_6S_6)	2-V (S_1S_7)	2-VI (S_6S_7)
				Number	*Number*	*Number*	*Number*	*Number*	*Number*
"Recovered" 2-I	S_4S_5	2-31	♀	0	42	33	47	43	41
			♂	37	43	49	9	35	38
2-II	S_6S_7	2-124	♀	34	0	39	(32)	33	39
			♂	33	0	44	32	35	32
2-III	S_4S_6	2-128	♀	0	28	0	-----	30	39
			♂	40	38	3	23	25	25
2-VI	S_6S_7	2-129	♀	45	40	20	24	47	0
			♂	27	49	31	39	39	0

[1] Values enclosed in heavy black lines are incompatible crosses.

As stated, a few seeds were obtained from the incompatible crosses or selfs made with 10 flowers per head in the greenhouse, but considerably more were obtained from manipulation of entire heads, either in greenhouse or field. Representative individuals from these seeds were selected to be tested for homozygosity in the greenhouse by backcrossing to the P and F_1 groups. The reaction of 4 typical homozygous plants is shown in table 5. Altogether, 41 plants were included in this test; 12 were found to be homozygous, 18 heterozygous, and 11 contaminants. This is a lower proportion of contaminants than was obtained with self-incompatible plants (*1*). The contaminants had genotypes that could not be accounted for on the basis of their parentage and they probably arose from accidental cross-pollinations. It

should be noted that no similar contaminations were found in the F_1 or F_2 plants, indicating that the chances of error in compatible crosses are considerably less than with incompatible matings.

TABLE 5.—*Number of seeds set per 10 flowers when typical homozygous plants were backcrossed to P and F_1 groups*

Genotype	Plant No.	Parent group	Origin[1]	As female or male parent	Seeds set[2] after backcrossing to group—					
					2-I (S_4S_4)	2-II (S_6S_7)	2-III (S_4S_6)	2-IV (S_5S_6)	2-V (S_4S_7)	2-VI (S_5S_7)
					Number	*Number*	*Number*	*Number*	*Number*	*Number*
S_4S_4	2-77	III	A	♀	0	34	42	33	48	42
				♂	0	38	0	34	0	41
S_5S_5	2-62	IV	B	♀	51	(51)	42	49	52	36
				♂	53	54	37	1	35	0
S_6S_6	2 54	IV	C	♀	15	15	9	14	16	25
				♂	53	0	0	0	29	14
S_7S_7	2-52	VI	D	♀	45	45	41	40	37	21
				♂	46	0	44	30	0	1

[1] A = Self-pollination under bag in the field; B = entire head manipulated in greenhouse, C = selfing 10 flowers without emasculation in greenhouse; D = selfing 10 flowers after emasculation in greenhouse.
[2] Values enclosed in heavy black lines are incompatible crosses.

Additional proof of the assumption that 2-I had changed from S_4S_5 to S_4S_4 between the time of the original cross and the time of the F_1 backcrosses was obtained from the F_2 and homozygous plants. The F_2 plant 2-31 (table 4) is typical of the "recovered 2-I" (S_4S_5) genotypes inasmuch as it was incompatible as a female but compatible as a male with the original female parent. On the other hand, all 2-III plants in the F_2 failed not only reciprocally with group 2-III but also as female with group 2-1 (table 4). The plants homozygous for S_4 failed in both directions with 2-I instead of only as males, and the S_5S_5 plants were compatible as male with 2-I when it was expected that they would be incompatible (table 5). Similar results were obtained when one F_3 progeny was tested. The seed used was that obtained from backcrossing the "recovered" S_4S_5 genotype as male onto the clones of the original female parent 2-I (table 4). When 10 of the F_3 plants were crossed reciprocally with plants of the "recovered" S_4S_5 genotype, all matings proved incompatible. These same plants, however, were compatible as males but incompatible as females with the original female parent 2-I. The anomalous behavior of all these plants, involving the failure of certain matings when they were expected to be compatible and the compatibility of others when they were expected to fail, can be explained in the same way as the incompatible F_1 backcrosses, simply by supposing that the clone used as 2-I in these tests was S_4S_4 instead of S_4S_5.

GENETICS OF PSEUDO-SELF-COMPATIBILITY

In both 1939 and 1940 the average seed set of the F_1 population was intermediate between those of the two parents (table 1). Also, in both years, a wide range was obtained in the averages of the individual F_1 plants. When the entire F_1 population (23 plants) was bagged in 1939, an average of 10.4 seeds per head was obtained

(table 6), while the individual plant averages ranged from 0.9 to 36.1. In 1940, only 9 F_1 plants were bagged, but their averages ranged from 3.0 to 48.4 seeds per head. Seven of these plants were selfed both years, and there was a significant interannual correlation ($r=0.867$, $P=0.02-0.01$) between the two groups of averages. It should be noted that at least one of the F_1 plants approached the pseudo-self-compatible parent in ability to set seed.

TABLE 6.—*Seed set in field of F_1, F_2, and backcross generation plants*

Cross	Generation	Percentage of plants with indicated number of seeds per head											Total number of plants	Mean number of seeds and standard deviation
		0-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50	50+		
2-1×2-2	F_1	26	30	17	9	13	...	...	4	...	...	...	23	10.4±1.9
2-14×2-12	F_2	76	20	4	...	...	...	...	...	...	...	...	25	3.1±.6
2-14×2-6	F_2	61	32	7	...	...	...	...	...	...	...	...	28	4.2±.6
2-14×2-8	F_2	64	12	10	6	4	2	2	...	...	...	...	50	6.3±1.1
Total	F_2	66	19	8	3	2	1	1	...	...	...	...	103	4.9±.6
2-1×2-12	B	37	20	15	9	7	7	2	4	...	...	...	46	11.0±1.4
2-1×2-14	B	64	18	5	5	5	...	...	...	5	...	...	22	7.1±2.0
2-1×2-6	B	71	21	4	...	...	4	...	...	...	...	...	28	4.8±1.2
2-1×2-8	B	22	30	4	22	7	7	...	4	...	...	4	27	13.9±2.5
Total	B	46	22	8	9	5	4	2	2	1	0	1	123	9.5±.9

The four F_1 plants used as parents of the F_2 generation showed considerable difference in seed-setting ability. Since one F_1 plant was common to the three F_1 intercrosses, any differences in the F_2 populations might be attributed mainly to the nonrecurrent F_1 plants. When the significance of the difference between means of the separate F_2 populations was measured by the t test, values exceeding those where $P=0.05$ were obtained in one case. The averages of the three F_2 populations appear to be related to the average of their respective nonrecurrent F_1 parent, and a highly significant difference was found between the average of the entire F_2 population and that of the F_1.

By the t test, highly significant differences between the individual backcross populations were found. Also, the difference between the average of the total backcross population and of the entire F_2 population was highly significant, but the difference between the average of the backcrosses and of the F_1 was not significant. More plants setting a large number of seeds per head, like the original pseudo-self-compatible parent, were recovered in the backcross progeny than in the F_2. From the frequency distributions and standard errors in table 6 it can be seen that large variations between the individual plant averages were found in every population and that all distribution curves were positively skewed. All these data suggest that pseudo-self-compatibility is a quantitative character, probably determined by several additive genes, some of which were heterozygous in the parents.

RELATION BETWEEN PSEUDO-SELF-COMPATIBILITY AND CROSS-INCOMPATIBILITY

Almost all F_1 and F_2 plants whose incompatibility groups were determined in the greenhouse were selfed under bag in the field. These comparative data for the F_1 are shown in table 1. Plants of both relatively high and low pseudo-self-compatibility were found in each of the four F_1 groups. It might appear that the plants in groups 2-IV and 2-VI, both of which bear S_5, were higher in pseudo-self-

compatibility than those in groups 2–III and 2–V, neither of which bears S_5. This relationship did not hold, however, in the F_2. Three plants setting over 10 seeds per head were found in group 2–VI, but no such plants were obtained in the recovered 2–I group, which also bore S_5. Apparently little if any relationship exists between the S factors and the amount of pseudo-self-compatibility.

NUMBER OF SEEDS PER POD

It was previously concluded (*1*) that the number of ovules per ovary is a heritable character that may have practical importance. Additional information on this subject has been obtained with these pseudo-self-compatible plants. The number of seeds per pod resulting from controlled cross-fertilization of 10 flowers per head was used as a measure of the number of ovules. Probably the most reliable data are shown in table 1, where the largest numbers of crosses per plant are recorded and where almost all crosses were made originally in duplicate. The original female parent, 2–1, averaged 44.6 seeds per head, while the male parent, 2–2, averaged 53.7. The F_1 averaged 40.8, and individual plants ranged from 32.8 to 46.7. When 4 of these clones, together with the 2 parental plants, were used as female in the F_2 and homozygous tests, an interannual correlation ($r = 0.938$, $P < 0.01$) was observed in their average seed-setting ability. Few crosses were made in duplicate in the F_2, so the averages of these plants are probably not as accurate as those of the F_1, but in general the averages of the different F_2 populations appeared to be related to the average of their nonrecurrent F_1 parent. The data indicate that this character is probably determined by several genes for which the parents were heterozygous.

DISCUSSION

The literature dealing with incompatibility has been reviewed recently by Stout (*7*). According to his terminology, the kind of cross-incompatibility observed here is determined by oppositional allelomorphs of the diploid personate type. This sort of inheritance has been reported for many species, but in most cases the plants have been self-incompatible rather than pseudo-self-compatible. Since this pseudo-self-compatibility was expressed only when the heads were manipulated, cross-incompatibility could be studied in these plants as easily as in self-incompatible individuals. The study was facilitated because the differences in seed set between compatible and incompatible crosses were generally distinct and because in every case the reflexing of the pedicels provided a check on the seed set.

Here, as with the self-incompatible plants of white clover (*1*), a disomic segregation among the S factors was observed. White clover, however, is a tetraploid with 32 somatic chromosomes. The original male parent in this study was included among the plants studied at meiosis (*5*), where 16 bivalents were found regularly, and root tips from representative F_1 plants all showed 32 chromosomes. Root tips from the 2–1 clone, which apparently had changed to S_4S_4, were examined cytologically, but no irregularities in its 32 chromosomes were observed. These observations support the previous conclusion (*1*) that the oppositional allelomorphs causing cross-incompatibility are present in only 1 of the 2 genomes found in white clover.

Four allelomorphs were found in the self-incompatible plants studied

for cross-incompatibility (*1*), and three additional factors were found in the parent plants used in this study. These seven genes have been obtained in homozygous condition, and all have been identified. It appears significant that seven of the eight genes borne by the four unrelated parents were different. The factor S_4, common to the two series, occurred in one case in a plant from Maryland seed and in the other in a plant isolated clonally from a pasture in central Pennsylvania. The evidence obtained in these investigations, together with that from several other sources, has led to the conclusion that a great many allelomorphs must be present in the series conditioning cross-incompatibility in white clover. In breeding experiments in which unrelated plants were used, the probability of cross-incompatibilities has been found to be very low. If the plants had been closely related, however, incompatibilities would have occurred much more frequently. It has been observed in other studies that the inbreds from pseudo-self-compatible plants may be homozygous for S factors. An F_1 between two such inbred lines might consist of a single intrasterile, interfertile group, in which case subsequent inbred generations would have to be derived by selfing rather than by intercrossing.

Several causes for pseudo-self-compatibility have been postulated—see review by Stout (*7*)—but in most plants it apparently is conditioned primarily by environmental conditions rather than by hereditary factors. In these plants of white clover, a complex hereditary mechanism for pseudo-self-compatibility has been demonstrated, but this ability to set selfed seed is apparently modified by the environment. Pseudo-self-compatibility is evidently not a case of "reduced seeding" in self-compatible plants, although part of the variation must be attributed to a reduction in seed set due to environmental conditions.

Pseudo-self-compatibility may be useful in a breeding program. Inbred lines may be obtained that tend to outcross instead of self when subjected to open-pollination. In this case, hybrid seed would be produced very easily. (This phase of the problem is now being investigated.) Likewise, if the F_1 consisted of a single genotype as the result of crossing two inbreds homozygous for five factors, sib mating would be prevented among the F_1 and four-way hybrids could be produced easily. In some respects, obtaining inbreds by means of pseudo-self-compatibility might be more desirable than using an S_f factor (*2*), since the latter might necessitate selecting only plants heterozygous for S_f in order to recover self-incompatible individuals at the end of the inbreeding. The usefulness of the type of pseudo-self-compatibility described will also depend on the ease with which it can be transmitted and selected for, but the present investigation only partially answers these questions.

SUMMARY

The original female parent of the *Trifolium repens* plants used in this study set a large number of selfed seeds when entire heads were manipulated, but it failed to set seed when 10 flowers per head were self-pollinated by hand in the greenhouse. The male parent was self-incompatible.

The pseudo-self-compatibility of the female parent was transmitted to the F_1, F_2, and backcross generation plants; it appears to be conditioned by several genes, which were additive in effect and heterozygous in the parents.

Four intrasterile, interfertile groups of 4, 4, 3, and 2 plants, respectively, were found among the F_1 plants. The compatible F_1 intercrosses averaged 40.8 seeds per 10 flowers, whereas the incompatibles averaged only 0.18. Reflection of the pedicels also was used to distinguish compatible from incompatible crosses.

The diploid personate theory of oppositional allelomorphs explains these cross-incompatibilities if it is assumed that the parents differed in all four factors which they carried.

When a further test was made by backcrossing 38 selected F_2 plants to the two parental and four F_1 groups, only the expected groups were obtained. In this way a certain genotype was assigned to each group, and the oppositional-factor hypothesis was confirmed for these pseudo-self-compatible plants.

Out of 41 plants resulting from incompatible crosses or selfs, 12 proved homozygous, 18 heterozygous, and 11 contaminants. Among the 12 were the 4 possible homozygous genotypes. One of the S genes from the pseudo-self-compatible parent was identical with the S_4 previously isolated from a self-incompatible plant.

When the original female parent was backcrossed with the F_1, F_2, and homozygous plants, certain combinations failed where they were expected to be compatible, and others were compatible where they were expected to fail. All of these results are explicable if it is assumed that this original parent changed from S_4S_5 to S_4S_4 subsequent to the original cross. This change could have resulted from either mutation or contamination in the field.

Since plants of both relatively high and low pseudo-self-compatibility were found in each of the six groups, it is evident that little relationship exists between the S factors and those conditioning the ability to set some selfed seed after manipulation.

It is pointed out that pseudo-self-compatibility may be useful in a breeding program.

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RELATIVE SUSCEPTIBILITY TO PYTHIUM ROOT ROT OF TWELVE DENT CORN INBREDS [1]

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INTRODUCTION

This paper presents the results of 2 tests of the relative susceptibility to pythium root rot (*Pythium arrhenomanes* Drechs.) of 12 inbred lines of dent corn (*Zea mays* L.).

Pythium arrhenomanes was first described by Drechsler [2] in 1928 as causing root rot of corn in Wisconsin and Illinois. The disease was first referred to by Johann et al.[3] in 1926 and more fully described in 1928.[4] The recent work of Rands and Dopp [5] has shown that the strains of *Pythium* that cause root rot of corn in Wisconsin, Illinois, Kentucky, Missouri, and other parts of the Corn Belt; root rot of wheat (*Triticum aestivum* L.) and other cereals in Canada; of pineapple (*Ananas sativus* Schult.) in Hawaii; and of sugarcane (*Saccharum officinarum* L.) in the southern United States, the Philippines, Hawaii, and other countries, all belong to one species, *P. arrhenomanes*, which is apparently a fungus of wide host range and of almost universal distribution.

Recent reports of root rot in the field and specimens of inbred and open-pollinated field corn from Iowa and Nebraska, from which *Pythium arrhenomanes* was isolated, have indicated that this root rot continues to have an important effect on the yield of field corn. Because of the wide distribution of this fungus and the rapidly increasing acreage of hybrid corn throughout the Corn Belt, it becomes increasingly important to learn more of the relative susceptibility to pythium root rot of the inbred parent lines in use at the present time.

The results of two greenhouse tests are given here as a measure of possible field damage.

MATERIAL AND METHODS

Seed [6] of 12 inbred lines of dent corn was washed for 10 minutes in a 1 : 1,000 solution of bichloride of mercury ($HgCl_2$), thoroughly rinsed in distilled water, and germinated on moist filter paper in Petri dishes. Each of the young seedlings was transferred to a 6-inch pot coated with asphaltum, and was allowed to grow 4 to 6 inches high

before inoculations were made. By inoculating the plants after they were several inches high, seedling injury was avoided. In order to eliminate some of the sources of variability and to obtain more or less reproducible results, the plants were grown in clean quartz sand that had been heated for 6 hours at 15 pounds' pressure. The plants were watered once or twice a week with a nutrient solution containing the following salts in the partial volume (molecular concentration) indicated: Ammonium nitrate (NH_4NO_3), 0.004; monopotassium phosphate (KH_2PO_4), 0.0015; dipotassium phosphate (K_2HPO_4), 0.0005; magnesium sulfate ($MgSO_4$), 0.001; and calcium chloride ($CaCl_2$), 0.003; and trace elements (p. p. m.): Manganese (Mn), 5; aluminum (Al), 0.56; zinc (Zn), 0.25; boron (B), 0.53; copper (Cu), 0.25; and bromine (Br), 0.26. Before sterilization, iron was added to the quartz sand in the form of iron magnetite sand, 1 percent by weight. Greenhouse temperatures were maintained as low as would permit of good growth, and between the additions of nutrient solution the sand was kept moist by watering.

Plants of the 12 inbred lines of dent corn were inoculated in the greenhouse at the Arlington Experiment Farm, Arlington, Va. The culture of *Pythium arrhenomanes* [7] used in making the inoculations had been isolated in August 1939 from an open-pollinated variety, St. Charles White, from York, Nebr. The diseased plants were 4 to 5 feet in height and showed typical rotting of the smaller roots.

There were 10 controls of each inbred in each of the tests and 10 replicates of each were inoculated. The plants were set at random in the greenhouse benches, the controls on one bench and the inoculated plants on a parallel bench. Inoculations were made by placing squares (1 cm.²) of corn meal-carrot agar cultures in the sand on either side of the plant. Sterile media were used for the controls. Measurements of the height of individual plants were made the day the plants were inoculated and every seventh day thereafter. At the end of the experiment the tops of the plants were cut off just above the roots and weighed. The roots were washed free of sand and weighed.

RESULTS

In the 1940 test, the corn plants grew for 4 weeks after inoculation with *Pythium arrhenomanes*. Figure 1 shows the differences in size at the end of the experiment of inoculated and uninoculated plants of typical inbreds.

Figure 2, *B*, shows the extent of root damage in the inbred Ia. L317 (fig. 1, *A*), which was the most resistant to root rot; and figure 3, *B* and *C*, the root damage in the inbred C. I.[8] 1 (fig. 1, *F*), which was the most susceptible. In these inoculated plants, the fine roots have been destroyed and even the larger roots show rotting. The root systems of the inbreds not illustrated also showed abundant fine roots in the controls and almost none in the inoculated plants. Because of high temperatures, the experiment was terminated after 4 weeks.

In the 1941 inoculation experiment, the plants grew for 7 weeks after inoculation. Differences were greater between uninoculated and inoculated plants, as shown in figure 4. But Ia. L317 continued to

[7] Identified by Charles Drechsler, of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.
[8] C. I. refers to accession number of the Division of Cereal Crops and Diseases. Formerly accession numbers for the inbred lines developed in this Division were designated by the prefix "U. S." instead of "C. I.," but they have recently been changed to avoid confusion with the designation of U. S. hybrids.

be the most resistant to *Pythium arrhenomanes* and C. I. 1 the most susceptible. The contrast between the bench of control plants and the bench of inoculated plants (fig. 5) gives some idea of the differences in growth. In figure 5, *B*, inoculated plants of Ill. R4, next to the steam pipes, grew much larger and more normally than in other positions on this bench. This was due to the higher temperatures and more rapid drying of the sand in these pots, both of which conditions are unfavorable for the development of *P. arrhenomanes*.

In both experiments, *Pythium arrhenomanes* destroyed most of the fine roots of all the inbred lines. In the more resistant inbred lines,

FIGURE 1.—Dent corn inbreds grown in sand plus nutrient solution, uninoculated and inoculated with *Pythium arrhenomanes* April 1, and photographed May 1, 1940: *A*, Ia. L317; *B*, Ia. Bl 345; *C*, Ia. Mc 401; *D*, C. I. 540; *E*, Ind. Tr; *F*, C. I. 1. *a*, Uninoculated; *b*, inoculated.

more of the larger roots were developed than in the susceptible ones.

The results of the 2 inoculation experiments on all 12 inbreds are given in tables 1 and 2. Table 1 shows the mean weekly height of inoculated and control plants, beginning at the time of inoculation, and weekly differences in growth in each inbred. Graphs in figures 6 and 7 show the mean weekly values in 1941 for some of the inbreds, illustrated in figure 4. The fungus interfered much less with the growth of inbreds Ia. L317 and Ia. Mc 401 than with that of Ind. Tr and C. I. 1. Ia. L317 showed the least susceptibility to attack and C. I. 1 the greatest susceptibility. This is shown not only in growth

of tops but in green weight of tops and roots as compared with controls.

The weekly figures in table 1 are summarized in table 2, which gives the total growth in centimeters of inoculated and uninoculated plants of each inbred, and the differences in growth between the inoculated

FIGURE 2.—Roots of dent corn inbred Ia. L317 grown in sand plus nutrient solution: *A*, Uninoculated; *B*, inoculated with *Pythium arrhenomanes* April 1, 1940. Photographed May 2, 1940.

and uninoculated plants of each inbred, in centimeters and in percentages of control plants. In column 1, all 12 inbreds show some reduction in growth owing to inoculation with *Pythium arrhenomanes*, but the amount of decrease varies with the inbred. The inbreds are arranged in the order of increasing percentage reduction in growth in 1940 (column 3). In columns 2 and 5 these differences are expressed in centimeters as a measure of the resistance of the different inbreds to inoculation with *P. arrhenomanes*.

FIGURE 3.—Roots of dent corn inbred C. I. 1 grown in sand plus nutrient solution: *A*. Uninoculated; *B* and *C*, inoculated with *Pythium arrhenomanes* April 1, 1940. Photographed May 2, 1940.

FIGURE 4.—Dent corn inbreds grown in sand plus nutrient solution, uninoculated and inoculated with *Pythium arrhenomanes* February 3, and photographed March 25, 1941: *A*, Ia. L317; *B*, Ia. Bl 345; *C*, Ia. Mc 401; *D*, C. I. 540; *E*, Ind. Tr; *F*, C. I. 1. *a*, Inoculated; *b*, uninoculated.

FIGURE 5.—Twelve dent corn inbreds grown in sand plus nutrient solution, Arlington Experiment Farm. Planted January 21, 1941. *A*, Uninoculated; *B*, inoculated with *Pythium arrhenomanes* February 3, 1941. Photographed March 25, 1941.
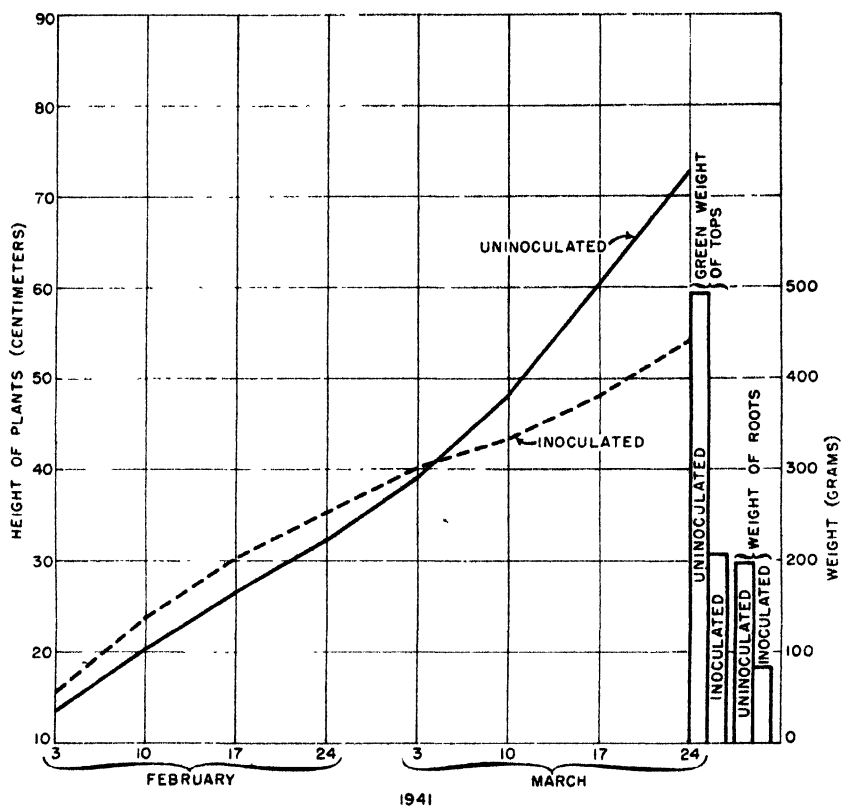
FIGURE 6.—Growth and green weight of tops and weight of roots of plants of dent corn inbred Ia. I.317, uninoculated and inoculated with *Pythium arrhenomanes*, Arlington Experiment Farm, 1941.
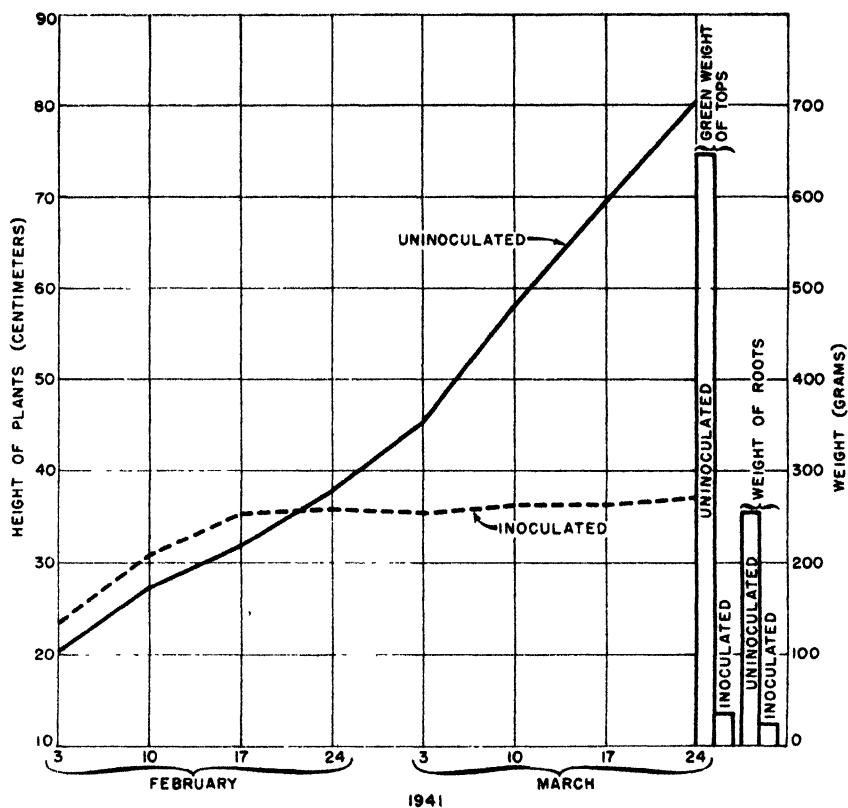
FIGURE 7.—Growth and green weight of tops and weight of roots of plants of dent corn inbred C. 1. 1, uninoculated and inoculated with *Pythium arrhenomanes*, Arlington Experiment Farm, 1941.

TABLE 1.—*Weekly increase in height of 12 inbred lines of dent corn not inoculated or inoculated with Pythium arrhenomanes Apr. 1, 1940, and Feb. 3, 1941*

Inbreds	Mean height of plants in—												
	1940: April—					1941							
						February-				March—			
	1	8	15	22	29	3	10	17	24	3	10	17	24
a. L317:	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm*
Not inoculated	21.6	31.8	45.9	57.9	71.5	13.6	20.2	26.7	32.3	39.0	48.3	60.7	72.9
Inoculated	21.9	32.1	44.8	53.3	62.3	15.5	23.8	30.7	35.4	40.1	43.2	48.1	54.4
Difference	− 3	− 3	1.1	4.6	9.2	−1.9	−3.6	−4.0	−3.1	−1.1	5.1	12.6	18.5
Ia. Bl 345:													
Not inoculated	20.5	29.9	41.9	54.2	65.8	14.6	23.0	30.0	34.1	41.4	51.2	61.3	68.3
Inoculated	20.8	30.1	39.4	47.8	54.7	17.6	26.9	32.2	34.2	35.9	37.4	39.0	41.9
Difference	− 3	−.2	2.5	6.4	11.1	−3.0	−3.9	−2.2	− 1	5.5	13.8	22.3	26.4
C. I. 5:													
Not inoculated	28.2	45.8	63.7	81.6	93.4	18.8	30.1	41.7	48.5	59.7	72.3	84.2	96.6
Inoculated	29.1	46.0	58.6	69.1	75.5	20.0	32.8	42.2	45.0	47.3	49.3	51.2	54.6
Difference	−.9	−.2	5.1	12.5	17.9	−1.2	−2.7	− 5	3.5	12.4	23.0	33.0	42.0
Ia. Mc 401:													
Not inoculated	24.9	39.3	54.9	72.8	85.0	15.1	24.7	35.1	40.8	50.3	62.6	74.8	87.0
Inoculated	26.1	40.6	51.8	60.8	68.5	19.4	30.6	40.1	44.2	47.0	51.6	55.3	59.6
Difference	−1.2	−1.3	3.1	12.0	16.5	−4.3	−5.9	−5.0	−3.4	3.3	11.0	19.5	27.4
Ky. 13:													
Not inoculated	30.7	42.0	56.4	71.9	84.8	21.1	31.6	39.4	43.9	49.8	59.6	73.4	86.7
Inoculated	31.1	42.9	50.1	58.8	67.4	25.7	35.8	41.3	44.2	45.9	49.8	52.9	58.8
Difference	− 4	− 9	6.3	13.1	17.4	−4.6	−4.2	−1.9	− 3	3.9	9.8	20.5	27.9
Ill. R4:													
Not inoculated	31.1	49.9	67.4	82.8	91.8	28.0	40.8	52.9	60.3	71.2	81.3	89.4	97.0
Inoculated	29.9	45.3	57.6	65.3	70.5	30.8	43.1	54.2	60.6	65.3	69.2	71.5	72.6
Difference	1.2	4.6	9.8	17.5	21.3	−2.8	−2.3	−1.3	− 3	5.9	12.1	17.9	24.4
Ia. L289:													
Not inoculated	26.3	35.6	54.2	70.1	84.5	19.5	28.7	36.8	42.6	50.3	62.3	74.7	84.5
Inoculated	26.4	37.1	48.7	56.4	64.4	20.6	31.1	36.4	37.6	37.8	40.1	41.8	44.6
Difference	− 1	−1.5	5.5	13.7	20.1	−1.1	−2.4	.4	5.0	12.5	22.2	32.9	39.9
Ill. Hy:													
Not inoculated	27.5	39.0	52.1	65.9	77.3	18.6	28.2	37.1	42.5	49.6	59.7	70.5	80.3
Inoculated	26.4	38.9	45.6	52.8	58.6	21.2	30.7	37.7	41.7	44.6	47.4	49.8	53.4
Difference	1.1	.1	6.5	13.1	18.7	−2.6	−2.5	−.6	.8	5.0	12.3	20.7	26.9
Ind. Tr·													
Not inoculated	22.3	37.0	54.8	73.1	86.3	21.1	32.2	43.9	53.9	67.3	80.4	91.1	98.1
Inoculated	21.0	36.6	44.2	53.0	60.5	24.4	35.4	43.1	48.4	50.6	53.0	54.0	55.0
Difference	1.3	.4	10.6	20.1	25.8	−3.3	−3.2	8	5.5	16.7	27.4	37.1	43.1
C. I. 4-8:													
Not inoculated	21.4	34.3	47.5	64.3	86.3	23.7	35.9	45.5	52.9	62.7	74.1	85.0	94.1
Inoculated	23.4	36.0	46.1	54.4	60.5	27.5	39.5	49.1	52.6	53.8	55.1	55.8	57.1
Difference	−2.0	−1.7	1.4	9.9	25.8	−3.8	−3.6	−3.6	.3	8.9	19.0	29.2	37.0
C. I. 540:													
Not inoculated	18.7	26.5	37.6	50.4	65.4	18.2	24.7	29.6	33.4	39.7	48.8	59.7	72.9
Inoculated	20.8	31.2	34.9	38.6	42.1	20.0	27.7	31.2	33.6	33.9	35.1·	36.0	37.9
Difference	−2.1	−4.7	2.7	11.8	23.3	−1.8	−3.0	−1.6	−.2	5.8	13.7	23.7	35.0
O. I. 1:													
Not inoculated	20.3	30.3	45.0	61.0	76.5	20.6	27.3	31.9	37.9	45.8	58.0	69.7	80.4
Inoculated	22.1	31.3	33.0	38.0	34.6	23.6	30.9	35.1	35.8	35.5	36.2	36.1	37.1
Difference	−1.8	−1.0	12.0	23.0	41.9	−3.0	−3.6	−3.2	2.1	10.3	21.8	33.6	43.3

TABLE 2.—*Results of sand-culture inoculation tests of resistance of 12 inbred lines of dent corn to Pythium arrhenomanes root rot*

	Mean increase in height of plants in—						Green weight of tops in—				Weight of roots in—			
	1940			1941			1940		1941		1940		1941	
Inbred	Total	Difference between control and inoculated	Difference ± controls	Total	Difference between control and inoculated	Difference ± controls	Total	Difference ± controls	Total	Difference ± controls	Total	Difference ± controls	Total	Difference ± controls
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)
	Cm	*Cm*	*Percent*	*Cm*	*Cm*	*Percent*	*Gm*	*Percent*	*Gm*	*Percent*	*Gm*	*Percent*	*Gm*	*Percent*
Ia. L317:														
Not inoculated	49.9	9.5	−19.0	59.3	−20.4	−34.4	343.9	−41.1	494.0	−58.1	271.0	−24.0	198.0	−58.6
Inoculated	40.4			38.9			205.8		207.0		206.0		82.0	
Ia. Bl 345:														
Not inoculated	45.3	11.4	−25.2	53.7	−29.4	−54.7	482.4	−44.2	500.0	−77.7	220.0	−32.7	157.5	−84.8
Inoculated	33.9			24.3			269.3		111.5		148.1		24.0	
C. I. 5:														
Not inoculated	65.2	18.8	−28.8	77.8	−43.2	−55.5	641.2	−51.6	848.0	−79.0	334.5	−35.5	243.0	−71.2
Inoculated	46.4			44.6			310.1		178.0		215.6		70.0	
Ia. Me 401:														
Not inoculated	60.1	17.7	−29.5	71.9	−31.7	−44.1	500.0	−44.0	537.0	−68.1	332.5	−42.9	200.0	−70.0
Inoculated	42.4			40.2			280.0		171.5		190.0		60.0	
Ky. 18:														
Not inoculated	54.1	17.8	−32.9	65.6	−32.5	−49.5	490.6	−51.5	562.5	−67.4	297.7	−35.8	284.0	−74.1
Inoculated	36.3			43.1			233.3		183.5		191.1		73.5	
Ill. R 4:														
Not inoculated	60.7	20.1	−33.1	69.0	−27.2	−39.4	625.1	−63.2	901.0	−61.1	321.1	−62.6	411.0	−68.0
Inoculated	40.6			41.8			230.0		350.2		120.0		131.5	
Ia. L289:														
Not inoculated	58.2	20.2	−34.7	65.0	−41.0	−63.1	488.8	−57.9	501.2	−89.4	271.1	−54.7	172.0	−85.5
Inoculated	38.0			24.0			205.7		53.2		122.8		25.0	
Ill. Hy:														
Not inoculated	49.8	17.6	−35.3	61.7	−29.5	−47.8	444.1	−56.2	479.1	−70.1	272.8	−54.4	122.5	−49.4
Inoculated	32.2			32.2			194.6		143.2		124.5		62.0	
Ind. Tr:														
Not inoculated	64.0	24.5	−38.3	77.0	−46.4	−60.3	463.5	−68.0	885.2	−86.0	262.2	−60.2	470.0	−88.0
Inoculated	39.5			30.6			148.5		124.0		104.3		56.2	
C. I. 4–8:														
Not inoculated	64.9	27.8	−42.8	70.4	−40.8	−58.0	448.0	−65.4	757.0	−82.2	245.0	−60.4	284.3	−81.9
Inoculated	37.1			29.6			155.0		135.0		96.9		51.5	
C. I. 540:														
Not inoculated	46.7	25.4	−54.4	54.7	−36.8	−67.3	292.7	−81.5	362.7	−91.1	189.0	−73.0	229.5	−87.8
Inoculated	21.3			17.9			54.2		32.3		51.0		28.0	
C. I. 1:														
Not inoculated	56.2	43.7	−77.8	59.8	−46.3	−77.4	510.0	−96.5	646.0	−94.5	284.5	−92.8	254.0	−91.3
Inoculated	12.5			13.5			17.9		35.5		20.6		22.0	

[1] Difference between differences required for significance, 16.3 cm

[2] Difference between differences required for significance, 9.5 cm.

Differences in growth of tops between inoculated and control plants of individual replicates were analyzed statistically[9] by the analysis of variance method, and the required difference for significance between mean differences was calculated for each of the two experiments. In

1940 this difference was $1.971\sqrt{\dfrac{2\times2\times171.51}{10}} = 16.32$ cm.; and in

1941, $1.971\sqrt{\dfrac{2\times2\times57.50}{10}} = 9.45$ cm.

The difference between Ia. L317, the most resistant inbred, and C. I. 1 was significant in each year. In 1941, in the longer test, there
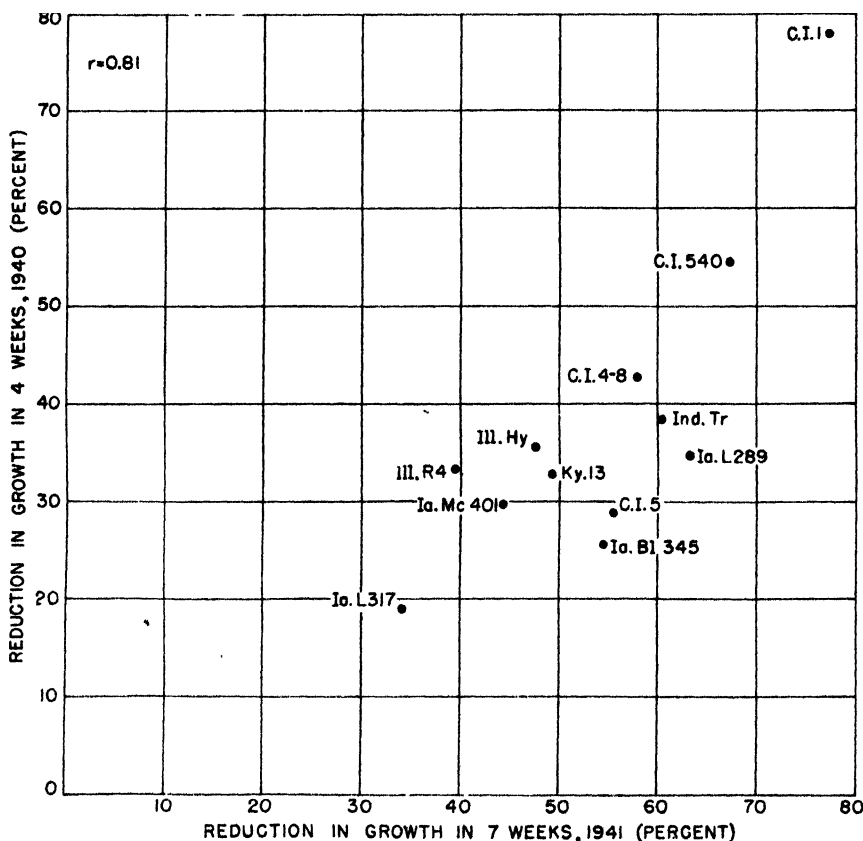
FIGURE 8.—Scatter diagram of percentage reduction in growth of dent corn inbreds inoculated with *Pythium arrhenomanes*, 1940 and 1941.

were significant differences between Ia. L317 and all other inbreds except Ia. Bl. 345, Ill. R4, and Ill. Hy.

In both years, inbred Ia. L317 was more resistant to root rot than any other inbred and C. I. 1 was more susceptible than any other. Inbred Ia. L317 is one of the most widely used inbreds in the development of hybrids in the Corn Belt. Although the results are not iden-

tical for the two tests, this is at least partly due to differences in environmental conditions difficult to control. According to other workers,[10] root rot due to *Pythium arrhenomanes* develops most abundantly under cool moist conditions. In the second experiment, the steam pipes undoubtedly had an influence on the inoculated plants of Ill. R4 and possibly other inbreds that were next to the pipes.

Percentage reductions in growth of tops (columns 3 and 6) for 1940 and 1941 are compared in a scatter diagram (fig. 8). Variability about the line of regression is an indication of the interaction between environment and strain in the two tests.

It should be noted that these are the results of inoculations with one strain of *Pythium arrhenomanes* isolated from root rot in dent corn growing under field conditions in one section of the Corn Belt. It is possible that there may be other strains of this fungus in other localities that may be more or less virulent than the strain used. Rands and Dopp[11] already have found that there may be wide differences in virulence in isolates of *P. arrhenomanes* from sugarcane in the same field and that populations may vary from season to season. It is possible that this may be true also for the fungus on corn.

SUMMARY

Twelve inbred lines of dent corn were tested for susceptibility to root rot by inoculation with *Pythium arrhenomanes*. The plants were grown in sand, watered with nutrient solution, and inoculated by adding agar cultures of the fungus to the sand after the plants were well established. Weekly measurements of growth of inoculated and uninoculated plants and weights of tops and roots showed that some inbreds were susceptible to root rot, some were resistant, and others were intermediate in reaction. Inbred Ia. L317 was the most resistant and inbred C. I. 1 the most susceptible. The fungus interfered to some extent with the growth of all the inbreds by destroying the fine feeding roots.

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[10] See footnote 5.
[11] See footnote 5.

RESERVE FOODS IN THE ROOTS OF WHITEWEED (CARDARIA DRABA VAR. REPENS)[1]

By C. G. Barr [2]

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INTRODUCTION

The food reserves in the roots of perennial weeds have been studied by a number of investigators who have assigned important roles to various carbohydrate fractions in attempts to determine the most effective methods of weed control. Bakke, Gaessler, and Loomis [3] found that sucrose and a dextrinlike compound or group of compounds are the characteristic reserves in bindweed. Barr [4] found that the reserves in the bindweed of his experiments consisted largely of sucrose and a polysaccharide fraction extracted with taka-diastase and commonly designated as starch. He did not, however, attempt to determine dextrin and starch separately.

The literature on the organic reserves in the roots of whiteweed is very limited.

Investigations of the reserve food in the roots of weeds were started at the Colorado Agricultural Experiment Station in 1935. The experiments discussed in this paper relate to the carbohydrate and nitrogen content of whiteweed roots as affected by various cultural practices and chemical treatments. All the experiments except as otherwise noted were conducted on land subject to irrigation but not irrigated during the course of the study.

MATERIALS AND METHODS

The material used for the experiments was the roots of whiteweed *Cardaria draba* (L.) Desv. var. *repens* (Schrenk) O. E. Schulz). Four separate types of collections were made to study the effects of cultivation and application of sodium chlorate on the root reserves:

(1) Root samples collected at biweekly intervals from April to December. Two series of plots were sampled, one in which the plants were undisturbed throughout the season, and the other in which the plants were clean-cultivated at 2-week intervals from April to October.

(2) Root samples collected at 4-day intervals for a period of 24 days (a) after a single cultivation in the spring, and (b) after small-grain harvest but without cultivation.

(3) Root samples collected from cultivated and undisturbed plots treated on various dates (tables 6 and 7) with dry sodium chlorate

at the rate of 3 pounds per square rod. Samples were taken at the time of treatment; viz, June 1, June 30, August 2, September 1, September 17, and September 30, and also 1 month after treatment. Checks were run on untreated and uncultivated plots.

(4) Root samples collected at the end of the growing season, about October 1, from plots (a) undisturbed; (b) cultivated weekly, April to October; and (c) cultivated biweekly, April to October.

COLLECTING AND ANALYZING SAMPLES

The method used in collecting the root samples, described in a previous report,[5] consisted in sifting the soil containing whiteweed roots and placing the roots with some soil in quart mason fruit jars for transport to the laboratory. A post-hole digger which made a hole 6 inches in diameter was used in digging the roots. By making four adjacent holes in the pattern of a square, a hole was produced 1 foot square and as deep as required. For the work on whiteweed, root samples were taken from both the first- and second-foot levels. Live roots only were taken, all dead and decaying portions such as were found in the cultivated plots being discarded. A cubic foot of soil was taken from each of five different locations in the plot to make up a standard composite field sample.

The roots were taken to the laboratory as soon as possible after digging, the soil and sand were removed, and the roots were washed with cold water. The excess water was removed by blotting with cheesecloth. Duplicate 50-gm. samples of the roots were weighed to ±0.05 gm. on a torsion balance. These were cut into pint mason fruit jars containing 200 ml. of boiling 95-percent alcohol, sealed, and allowed to simmer in a boiling water bath for 45 to 60 minutes. The samples were then stored until they could be conveniently extracted and analyzed.

The preserved root material was extracted by decantation with 80-percent boiling alcohol until reducing substances had been removed from the sample as determined by tests. From 10 to 14 extractions were required. The extract from each sample was collected in a volumetric flask and the extracting liquor made to volume after completion of the extraction. Two hundred milliliter portions of this liquor were used for the sugar determinations. The alcohol was removed by evaporation and the resulting water solution was cleared with neutral lead acetate. Reducing substances were determined, after invertase digestion, by reduction of alkaline potassium ferricyanide and titration of the reduced iron with standard ceric sulphate solution.

It should be pointed out that considerable trouble was encountered in making sugar determinations by the Munson-Walker method. During the heating of the water solution of sugars with Fehling's solution, a colloidal mass of reduced copper together with other interfering substances was produced which caused great difficulty in filtering. This precipitate was difficult to break up on the asbestos mat in the Gooch Crucible, and it was not entirely soluble in the ferric ammonium sulfate. Titration of the reduced iron with potassium permanganate gave results which disagreed as much as 100 percent when duplicate aliquots were run from the same sample. The source of error was shown to be in the precipitation of the copper, and in the

[5] BARR, C. G. ORGANIC RESERVES IN THE ROOTS OF BINDWEED. Jour. Agr. Res. 60: 391, 413, illus. 1940.

fact that the end point was unstable in the permanganate titration. Tests showed that both clearing and deleading were complete. Whether the difficulty was due to salts contained by the roots of whiteweed, extractable with boiling 80-percent alcohol, or to certain complex carbohydrate compounds peculiar to whiteweed or to both is not known. Osazone reactions after hydrolysis with dilute hydrochloric acid or invertase yielded a mixture of crystals resembling glucosazones, maltosazones, and other unidentified crystals which suggested the presence of trisaccharides.

Ceric sulfate was found to be suitable in the analysis of carbo-hydrates in the roots of bindweed and whiteweed,[6] hence, ceric sul-fate was used throughout this experiment in estimating the quanti-ties of reducing substances.

The residue, after the removal of the soluble carbohydrates, was dried to constant weight at 100° C., ground to suitable fineness, and analyzed for starch and acid-hydrolyzable materials separately.

For the starch determinations, 1-gm. samples of the residue were gelatinized and digested with undiluted taka-diastase until negative iodine tests for starch were obtained. The digest was then filtered and washed, the filtrate was cleared with neutral lead acetate, and the glucose equivalent after hydrolysis was determined on aliquots. It has been pointed out in a previous paper [5] that in the determination of starch by this method, small amounts of other substances that yield reducing substances upon hydrolysis may be brought into solution by the gelatinizing process and taka-diastase digestion. The technique, serves, however, to give an adequate estimation of the important reserve polysaccharides.

FORM OF FOOD RESERVES IN THE ROOTS OF WHITEWEED

Important food reserves in whiteweed occur in the form of sugar and starch. Only small quantities of reducing sugars were found, amount-ing to less than 1.50 percent in August and showing only moderate fluctuation. Determinations for reducing sugars were, therefore, not made on all the samples. Although other carbohydrate fractions, such as the dextrins and a group of compounds extracted with hot dilute mineral acids, do occur, the writer's experiments have shown that they probably play a less important role than the sugars and starch.

Whiteweed roots are unusually high in starch content and a cross section of a root appears not unlike the cross section of a potato. At the peak of accumulation the tissue is gorged with starch and an intense blue-black color is produced with iodine in potassium iodide solution over the entire section, indicating the vast amount of starch present. The moisture content of whiteweed root tissue varies from 70 to 90 percent, depending upon the environmental conditions under which the plant grows.

Results for the carbohydrate analyses were calculated as: (1) per-centage of fresh weight, (2) percentage of dry weight, and (3) percent-age of residual dry weight; that is, calculations were made on the dry weight of the tissue after the soluble carbohydrates and the poly-saccharides were removed. The residual dry-weight calculations were difficult to interpret and their meaning was of little value from the standpoint of a cultivation control program, and the dry-weight

[5] BARR, C. G. ORGANIC RESERVES IN THE ROOTS OF BINDWEED. Jour. Agr. Res. 60. 391, 413, illus. 1940
[6] BARR, C. G. APPLICATION OF THE CERIC SULPHATE METHOD IN THE ANALYSIS OF CARBOHYDRATES IN THE ROOTS OF LEPIDIUM AND CONVOLVULUS. Plant Physiol. 14: 285-296, illus. 1939.

calculations were so high and variable that they seemed worthless. The readily available carbohydrates are expressed in the tables, however, as percentage of both the fresh weight and the dry weight for comparison.

The value of the dry-weight data may be estimated from a study of table 1. Samples for the second-foot level on September 30 contained 2.04 percent total sugar and 21.14 percent starch, amounting to 23.18 percent readily available carbohydrates. It has been shown [6] that carbohydrates determined by the method used in this study are 1 to 5 percent higher than by the official Munson-Walker method. The percentages obtained here, therefore, are reasonably accurate, but assuming the actual readily available carbohydrate content to be about 20 percent, 20 gm. of every 100 gm. of fresh plant material would represent respirable material. The particular sample under consideration contained 72 percent of moisture, or 28 gm. of dry plant material. The quantity of readily available carbohydrates would, therefore, be over 80 percent on the dry-weight basis and would vary from 77 percent to 89 percent with a change of sample moisture content of ±2 percent. If, however, the data are calculated on the fresh-weight basis, a variation of ±2 percent in moisture content of the sample would result in a change of readily available carbohydrates from 21.50 percent to 24.81 percent. It seems desirable, therefore, to base interpretations on fresh weight.

TABLE 1.—*Seasonal trends in the carbohydrate content (percent) of whiteweed roots in the first- and second-foot levels of undisturbed and cultivated plants, 1936*

[The two sets of data for each sampling date are for the first- and second-foot levels, respectively]

| Date of sampling | Undisturbed plants | | | | | | | | | | | |
	Reducing sugars	Total sugars	Starch	Acid hydrolyzable substances	Readily available carbohydrates on bases of — Fresh weight	Dry weight	Reducing sugars	Total sugars	Starch	Acid hydrolyzable substances	Readily available carbohydrates on basis of — Fresh weight	Dry weight
Apr. 25	0.73	1.50	2 69	2 35	4.19	33 6						
	.63	2.82	1 64	2 13	4.46	35.06						
May 9	.56	1 72	2.07	3 92	3.79	33 33	0.67	1.38	1.65	2 71	3.03	22 80
	67	2.26	2.27	3 23	4.54	32.70	.66	2.79	3 96	1.72	6.75	61.38
May 25	78	2.15	7.41	3 52	9 56	48 72	.62	1.46	2 74	2 51	4.20	35.23
	.66	2.88	6 41	3 07	9.29	58.07	.66	2 68	3.81	2 27	6 49	59.55
June 8	.70	2.77	12 20	2 81	14 97	54 67	.50	1.00	4 39	3.43	5.39	47 11
	.80	3.95	13.80	3.19	17 75	75 77	60	1.60	3 78	2 43	5.38	55 57
June 22	.81	1.91	15.77	5.67	17.68	59.36	.44	.92	1.59	3 27	2.51	20 52
	.79	3.02	15 94	6.44	18 96	83 23	18	82	3 05	2.35	3 87	39.38
July 6	1.24	3.78	18.36	8.08	22.14	66.16	.55	1 42	3 03	2.75	4 45	42 20
	1.02	4.16	19 45	6.71	23.61	92 47	56	1.85	3 53	2 14	5.38	54.69
July 21	1.16	2.96	21.88	6 61	24.84	71 50	.71	2.11	4.54	2.59	6.65	53.20
	.93	3.86	20 50	4.91	24.36	95.52	.68	2 12	3.81	3 03	5.93	48.52
Aug. 2	1.41	2.77	21.24	4.74	24.01	68.57	.76	2.52	3.76	3.28	6.28	62.50
	1.28	2.88	22 43	4.33	25.31	71.70	.67	3.17	3.40	3.48	6.57	65.50
Aug. 18	1.32	2 20	16 80	5.25	19 00	58.82						
	1.33	2 99	19.68	3.84	22.67	74.53						
Sept. 1	1.16	1.98	17.04	4.96	19.02	54 81						
	1 04	2 96	20.19	3.18	23.15	76.55						
Sept. 13	1.06	2.16	15.38	5.05	17.54	55.15						
	.93	2.24	21.27	3.13	23.51	79.93						
Sept. 30		2.51	14.77	4.86	17.28	64.14						
		2.04	21.14	3.09	23.18	82.78						
Oct. 16		3.49	14.73	4.67	18.22	64.15						
		3.10	18.36	3.33	21.46	82.54						
Oct. 29		5.46	15.08	4.89	20.54	72.83						
		4.36	19.36	3.32	23.72	88.92						
Dec. 3		7.44	9.00	4.66	16.44	72.10						
		6.65	12.77	3.41	19.42	89.90						

[6] BARR, C. G. APPLICATION OF THE CERIC SULPHATE METHOD IN THE ANALYSIS OF CARBOHYDRATES IN THE ROOTS OF LEPIDIUM AND CONVOLVULUS. Plant Physiol. 14: 285-296, illus. 1939.

<div align="center">TREND OF ROOT RESERVES DURING THE SEASON</div>

Total sugars and starch show marked accumulation in the roots of undisturbed whiteweed plants from April to early summer. Roots from the second-foot level contained uniformly more total sugars than those from the first-foot level until about the middle of September, when the percentages were reversed (table 1, fig. 1). There was a gradual accumulation of sugars from April 25 to July 6, 1936, when a maximum of about 4 percent was reached. The initial accumulation was followed by a steady decrease until about the middle of September,
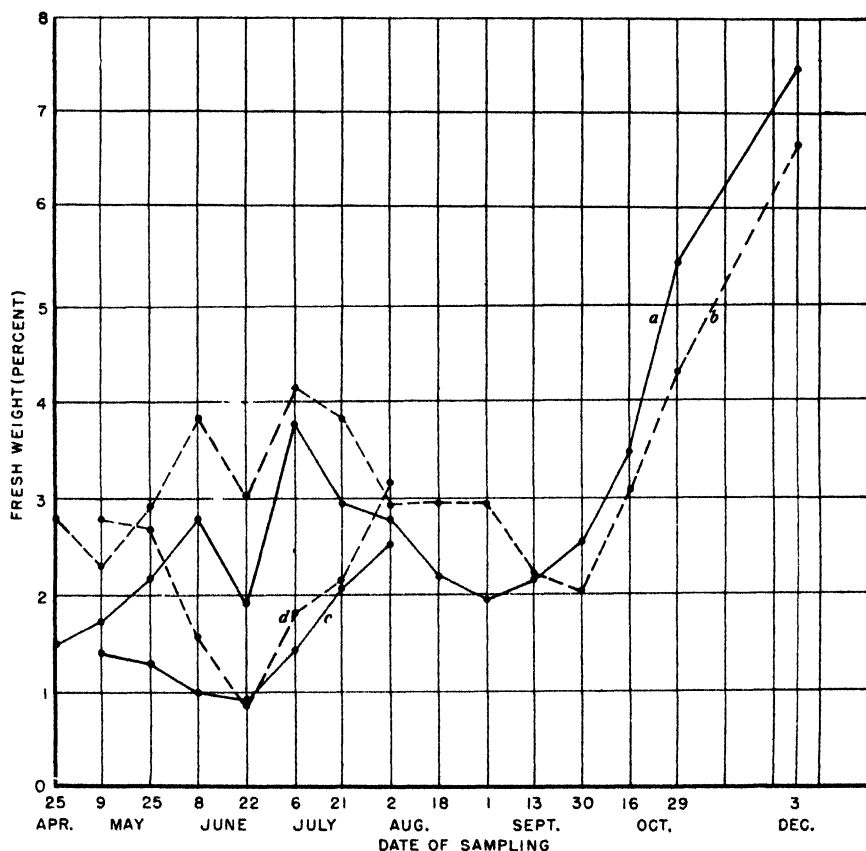
FIGURE 1.—Total sugar content of whiteweed roots when undisturbed and when cultivated at 2-week intervals, 1936: *a*, First foot, undisturbed; *b*, second foot, undisturbed; *c*, first foot, cultivated; *d*, second foot, cultivated.

after which the sugar content increased rapidly, reaching 6 or 7 percent by December 3 (fig. 1). The rapid increase in total sugar content after October 1 is interpreted as the result of the shift from polysaccharides to sugars that commonly occurs in plants as cold weather approaches. Evidence supporting this interpretation is the simultaneous reduction in the percentage of starch as the sugar content increased (compare figs. 1 and 2). The same observation has been made at this station in work on reserves in bindweed. A noteworthy point is the lag in the
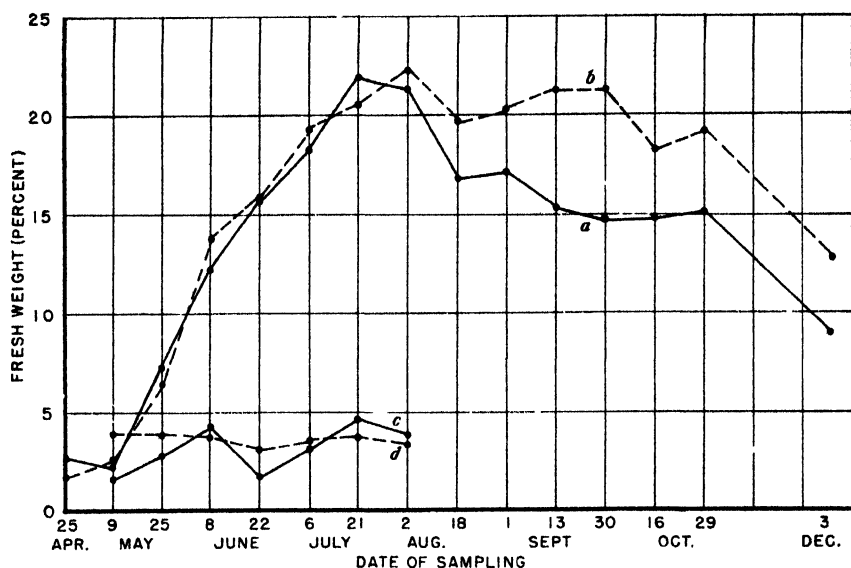
FIGURE 2.—Starch content of whiteweed roots when undisturbed and when cultivated at 2-week intervals, 1936: *a*, First foot, undisturbed; *b*, second foot, undisturbed; *c*, first foot, cultivated; *d*, second foot, cultivated.
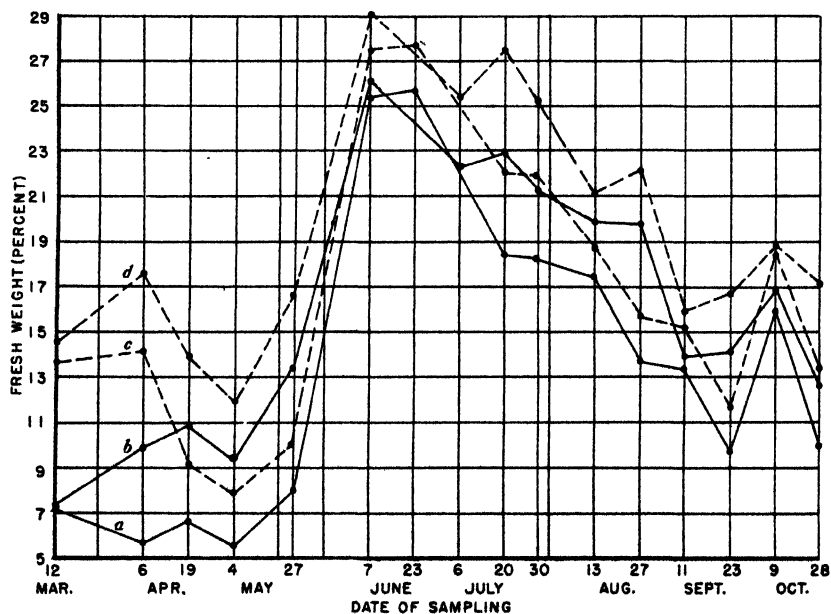
FIGURE 3.—Starch and readily available carbohydrate content of whiteweed roots undisturbed, 1937: *a*, Starch, first foot; *b*, starch, second foot; *c*, readily available carbohydrates, first foot; *d*, readily available carbohydrates, second foot,

maximum percentage of starch which occurred about 1 month later than the initial maximum total sugar content. An unexpected high value of over 20 percent (fresh-weight basis) of starch was found in the roots of undisturbed plants.

Plants that were clean-cultivated at 2-week intervals throughout the season failed to show a total sugar content greater than 3.17 percent, and cultivation held the starch to about 4.54 percent. Plants on the cultivated areas were rapidly disappearing by the last of June, and when samples were collected on July 6 considerable difficulty was encountered in finding sufficient roots to preserve for analysis. Many dead and decaying roots were found on this and the next two sampling dates, and no samples could be collected from the cultivated area after August 2. During the summer of 1937 only a very few scattered plants appeared on the clean-cultivated areas. Under the conditions of the experiments, therefore, 96 to 98 percent eradication was obtained by one season of clean cultivation.

It should be pointed out also that the infestation of whiteweed in the Fort Collins area was limited; plots were not available in 1937 for extended systematic cultivation studies and for this reason the data for 1937 are meager. Samples were collected, however, which yielded information on the seasonal trend of root reserves in undisturbed plants (table 2 and fig. 3).

TABLE 2.—*Seasonal trends in the carbohydrate and nitrogen content (percent) of whiteweed roots in the first- and second-foot levels of undisturbed plants, 1937*

[The two sets of data for each sampling date are for the first- and second-foot levels, respectively]

Date of sampling	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates on basis of --		Colloidal nitrogen	Soluble nitrogen
				Fresh weight	Dry weight		
Mar 12	6.44	7.21	4.19	13.65	61.22	0.429	0.293
	7.27	7.31	2.28	14.58	85.23	.176	250
Apr. 6	8.55	5.71	3.92	14.26	69.29	414	.278
	7.65	9.96	3.53	17.61	......	.192	.181
Apr. 19	2.35	6.77	4.03	9.12	44.75	.399	.234
	2.99	10.90	2.11	13.89	71.62	181	.150
May 4	2.36	5.61	3.74	7.97	38.08	.260	156
	2.36	9.52	2.65	11.88	61.36	138	107
May 27	2.13	8.04	5.09	10.17	36.79	.239	.066
	3.12	13.47	4.53	16.59	65.40	.173	.080
June 7	2.28	25.31	5.40	27.59	49.96	.254	087
	3.08	26.03	3.59	29.11	......	.174	100
June 23	2.08	25.67	5.66	27.75	74.14	384	.140
	3.80	--------	3.58	--------	......	.296	.137
July 6	2.13	--------	--------	--------	--------	126	.168
	3.07	22.33	1.90	25.40	71.10	.279	.145
July 20	3.70	18.40	3.21	22.10	56.46	468	.216
	4.56	22.94	4.64	27.50	69.57	.285	182
July 30	3.70	18.28	2.60	21.98	57.18	.485	.228
	3.97	21.24	2.31	25.21	63.93	365	.199
Aug. 13	1.26	17.42	7.67	18.68	50.29	505	.195
	1.16	19.86	5.91	21.02	58.64	.365	.164
Aug. 27	1.91	13.70	7.40	15.61	44.19	428	204
	2.31	19.79	5.31	22.10	63.43	.554	222
Sept. 11	1.77	13.38	7.80	15.15	45.09	.284	.221
	2.02	13.89	9.98	15.91	46.64	.408	162
Sept. 23	1.85	9.76	4.24	11.61	39.48	.320	.166
	2.44	14.18	2.35	16.62	57.96	.221	.166
Oct. 9	2.43	16.06	4.79	18.49	56.27	.539	.222
	1.90	16.91	2.46	18.81	55.33	.364	.193
Oct. 28	3.43	9.91	4.25	13.34	42.13	.486	262
	4.42	12.66	2.35	17.08	53.30	.338	.243

The observed increase in the sugar content of the roots of cultivated plants after June 22, 1936, suggests that translocation of soluble carbohydrates has taken place from lower to upper roots. The soluble carbohydrates in the lower roots would presumably be derived from complex carbohydrate reserves. It suggests further that the continual regeneration of new-shoot growth brought about by the frequent cultivations stimulated enzymatic activity which resulted in higher soluble-carbohydrate content at the expense of complex reserves in the deeper roots. Whiteweed root samples taken to a depth of 3 feet in 1935 showed progressive increases in total sugars and starch with increasing depths from which the roots were collected.

The reducing sugars and acid-hydrolyzable substances showed no important variations in either undisturbed or cultivated plants.

Owing to lack of material, it was not possible to make nitrogen determinations on the samples collected in 1936. Analyses were made,
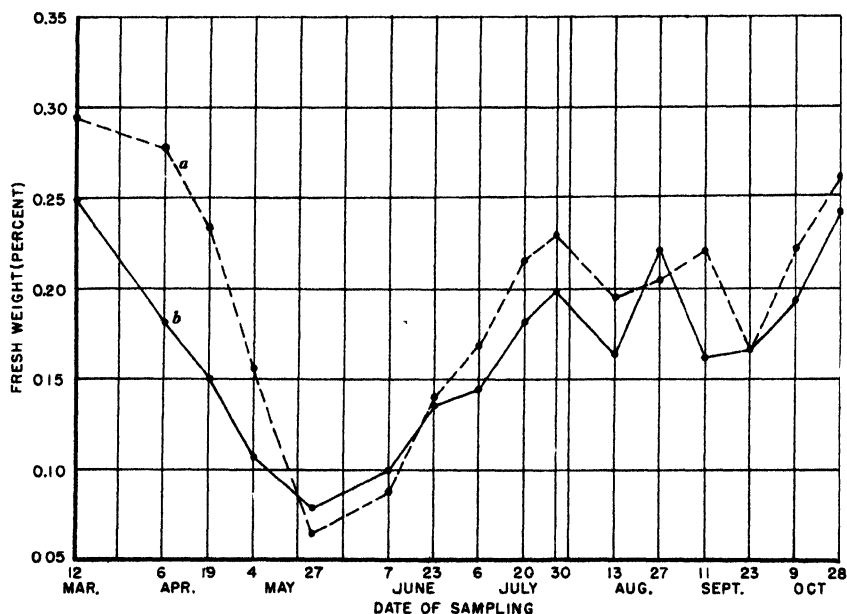
FIGURE 4.—Soluble nitrogen content of whiteweed roots when undisturbed, 1937: *a*, First foot; *b*, second foot.

however, for soluble and residual nitrogen in the roots collected in 1937. The data are given in table 2 and shown graphically in figures 4 and 5.

The marked decrease in soluble nitrogen from March until the latter part of May suggests the rapid utilization of that fraction in vegetative growth. Field notes taken during this time show that the plants exhibited considerable vegetative growth until the end of May when flowering was initiated. Mature seeds were observed by June 23 and the plants appeared to be going into a dormant condition. From June 23 until August 13 there was a period of vegetative inactivity and the soluble nitrogen content increased from about 0.07 percent from May 27 to over 0.20 percent by August 27.

A noteworthy point also is the relation between soluble nitrogen and carbohydrates during the period of vegetative growth in April and May. The elaboration of carbohydrates was apparently not sufficiently rapid to keep pace with the rate of their utilization in the formation of new protoplasts. The roots, being reserve sources of supply for the materials necessary for growth, showed a reduction of about 44 percent in carbohydrate content during the period from April 6 to May 4. The carbohydrates and soluble nitrogen each showed distinct accumulation after flowering, but the carbohydrates increased at a considerably more rapid rate than the nitrogen.

The fluctuation in the colloidal nitrogen content from March 12 to August 13 closely resembled that of the soluble nitrogen during the same period. This was especially true of the roots from the first foot of soil. Both the soluble and the residual nitrogen showed such peculiar
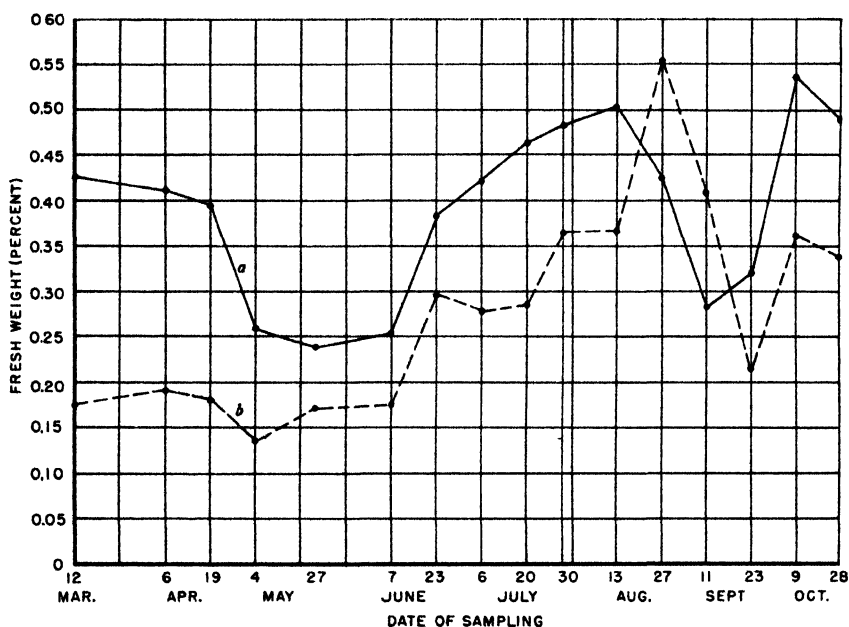
FIGURE 5.— Colloidal nitrogen content of whiteweed roots when undisturbed, 1937: *a*, First foot; *b*, second foot.

fluctuations after August 13 that it is difficult to attach any meaning to the variations.

A series of collections at 4-day intervals was started on July 21 after the harvest of wheat growing on whiteweed infested land. During the period from July 21 to August 14 the weather was uniformly cool; field notes show that the daytime temperatures were 70° to 76° F. No rain was recorded for this period and the plot was not cultivated and received no irrigation after the wheat harvest. Sampling on the area was continued until about the middle of August, but no important changes occurred in the carbohydrates under the conditions of the experiment. There was, however, an increase in the carbohydrate content as compared with that in the early-season collection. The data are given in table 3.

TABLE 3.—*Seasonal trends in the carbohydrate content (percent) of the roots of noncultivated whiteweed after wheat harvest, first- and second-foot levels, 1936*

[The two sets of data for each sampling date are for the first- and second-foot levels, respectively]

Date of sampling	Reducing sugars	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates on basis of—	
					Fresh weight	Dry weight
July 21	1.16	2.74	21.88	6.61	24.62	71.50
	.93	3.94	20 53	4.91	24 47	95.52
July 25	1.40	2.97	23.06	4.94	26.03	69.38
	1.15	3.31	27.45	3.90	30.76	92.40
July 29	1 34	2.58	21.36	5.64	23.94	68.91
	1.30	3.49	24.55	3 89	28.04	95.05
Aug. 2	1.41	2.77	21.24	4.74	24.01	68.57
	1.28	2.88	22 43	4.33	25.31	71.70
Aug. 6	1.16	2.37	18 09	5.41	20.46	62 18
	85	3 47	17.75	3.31	21.22	79.77
Aug. 10	1.34	2.32	18.85	5.26	21.17	61.87
	1.03	2 94	20.80	3.40	23.74	78.87
Aug. 14	1 29	2.34	20.18	4.89	22.52	62 43
	1.13	3.33	20.19	4.46	23.52	78 85

TREND OF ROOT RESERVES AFTER CULTIVATION

An area uniformly infested with whiteweed was cultivated on May 1, 1936, but not thereafter. Root samples were collected at 4-day intervals for a period of 24 days after the cultivation. Samples were taken at the same time from an adjacent control plot that had not been cultivated, but was otherwise similar. The plants on these plots emerged about April 15. On May 9 the undisturbed plants were 6 to 10 inches high and were 90 percent in full bloom; the undisturbed plants were in full bloom on May 16. The plants on the cultivated area emerged between May 9 and May 15.

The trend of the starch and readily available carbohydrates [7] in the roots from the top 2 feet of soil, undisturbed and cultivated, is shown in table 4. The data for only the first-foot level are plotted in figure 6 since the fluctuations in the sugar and starch content of the roots from the first foot of soil seemed to show the general trend for the second foot also.

Only a moderate increase in the carbohydrate content occurred in the roots of either the undisturbed or the cultivated plants during the first 12 to 16 days after cultivation. Twelve days after the beginning of the experiment there was a surprisingly rapid increase in the readily available carbohydrate content of the roots of the undisturbed plants. On May 25 the readily available carbohydrates were nearly 300 percent of their value on May 12. The low point in the carbohydrates for the cultivated plants was 4 days later than for the undisturbed plants, and although there was a distinct accumulation after 16 days, the readily available carbohydrate content on May 25 was about 176 percent of its value on May 16. Accumulation of reserves was delayed apparently by cultivation, but in spite of cultivation the plants showed a marked increase in root reserves after 16 days.

The experiment was repeated in 1937. The plot was cultivated on May 8 and again on May 17, when the first root samples were col-

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[7] The term "readily available carbohydrates" is used in this paper to express the sum of the total sugar and starch, commonly assumed to be readily available reserve materials.

lected.   Vegetative growth was limited by the low soil-moisture content but a few plants were emerging on May 22.   On May 31 there was 0.75 inch of rain and during the first 6 days of June there was a total of 1 inch of rain.   The results of the study in 1937 are similar to those obtained in 1936, as a comparison of the data in tables 4 and 5 will show.   In the 1937 experiment the accumulation started about 10 days after cultivation and the total carbohydrate content was increasing rapidly at the end of the experiment on June 7.

While it may not seem necessary to cultivate oftener than at inter-
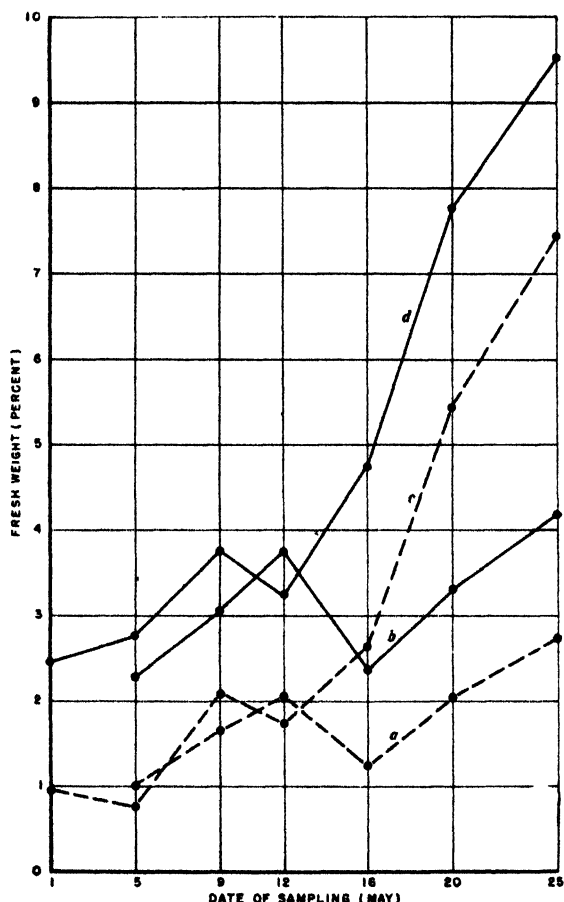
FIGURE 6.— Carbohydrate content of whiteweed roots first-foot level, after cultivation. Sampled at time of cultivation and at approximately 4-day intervals for a period of 24 days; *a*, Starch, cultivated; *b*, readily available carbohydrates, cultivated; *c*, starch undisturbed; *d*, readily available carbohydrates, undisturbed.

vals of 16 days, the data suggest that any delay beyond 16 days would permit rapid accumulation of reserves.   It is likely that the interval might be lengthened if a second or even a third cultivation should be made coincident with the start of reserve-food accumulation as indicated by the analysis of roots.

TABLE 4.—*Seasonal trends in the carbohydrate content (percent) of whiteweed roots after cultivation in the first- and second-foot levels, 1936*

[The two sets of data for each sampling date are for the first- and second-foot levels, respectively]

Date of sampling	Uncultivated					Cultivated May 1				
	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates on the basis of—		Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates on the basis of-	
				Fresh weight	Dry weight				Fresh weight	Dry weight
May 1....	1. 50	0 97	0 74	2 47	18. 98					
	2. 49	83	2 46	3. 32	27. 39					
May 5...... .	2. 02	. 76	2. 70	2. 78	17. 13	1. 21	1 00	3 06	2 21	14. 74
	2. 62	3. 43	2. 44	6. 05	43. 32					
May 9........	1. 72	2. 07	3. 92	3 79	23. 92	1. 38	1. 65	2 71	3 03	22 80
	2. 26	2. 27	3. 23	4. 53	32 70	2. 79	3. 96	1. 72	6. 75	61. 38
May 12.	1. 52	1. 74	3. 15	3. 26	22. 23	1. 72	2 02	2. 38	3. 74	29. 40
	2. 22	2. 36	2. 75	4. 58	32 52	2. 43	2 22	2. 07	4. 65	40. 34
May 16 . .	2. 06	2 65	3. 11	4. 71	32 33	1. 17	1. 21	2 51	2. 38	18 45
	2. 45	3. 70	2 10	6. 15	47 30	1. 74	2 00	2 19	3 74	32 81
May 20	2. 24	5. 46	4. 70	7. 70	37. 48	1 25	2 05	3 56	3. 30	26 05
	2 86	6. 23	3. 88	9. 09	54. 96	2 14	3. 22	3. 07	5. 36	44. 46
May 25....	2. 15	7 41	3. 82	9. 56	48. 72	1 46	2. 74	2 51	4. 20	48. 72
	2 88	6 41	3. 07	9 29	58. 07	2. 71	3. 82	2. 27	6 53	59. 55

TABLE 5.—*Seasonal trends in the carbohydrate content (percent) of whiteweed roots after cultivation, in the first- and second-foot levels, 1937*

[The two sets of data for each sampling date are for the first- and second-foot levels, respectively]

Date of sampling	Total sugars	Starch	Acid-hydrolyzable substances	Readily available carbohydrates on basis of—		Colloidal nitrogen	Soluble nitrogen
				Fresh weight	Dry weight		
May 17.....	1. 35	7. 71	3. 76	9. 06	43. 18	0. 220	0. 171
		11. 20	3. 06			. 144	. 127
May 22....	. 99	3. 61	2. 94	4. 60	22. 84	. 124	. 150
	1. 64	5 33	2. 33	6. 97	40. 84	. 133	. 117
May 27....	1. 49	5. 04	3. 90	6. 53	32. 47	. 226	. 145
	2. 40	8. 81	2. 24	11. 21		. 138	. 128
June 1	1. 05	3. 61	3. 67	4 66	24. 07	. 231	. 142
	1. 81	9. 82	2. 79	11. 63	75. 32	. 070	. 124
June 7.............	1. 53	9. 04	2. 97	10. 57	52. 53	. 176	. 144
	2. 48	13. 96	2. 46	16. 44	83. 53	. 124	. 134

EFFECT OF SODIUM CHLORATE ON ROOT RESERVES

The use of sodium chlorate in the control of noxious weeds is a common and successful practice. Recommendations vary, especially regarding the rate of application. Most workers agree that the best results are obtained when the chemical is applied in late summer or early fall.

Experiments were designed to study the root reserves of whiteweed as affected by a combined treatment of sodium chlorate and cultivation. Two series of plots which were uniformly infested with whiteweed were selected. One group of plots was cultivated three times,

May 1, May 12, and May 20, 1936; the other was left undisturbed. On June 1, and at irregular intervals thereafter (tables 6 and 7), root samples were taken from one plot each of the undisturbed and cultivated plants, and immediately thereafter dry sodium chlorate was applied to each sampled plot at the rate of 3 pounds per square rod. One month later root samples were again collected from the chlorate-treated plots and two new plots (one cultivated and one undisturbed) were sampled and treated with sodium chlorate as stated. The same system was followed throughout the season except in September, when samples were taken and treatments were made at 2-week intervals.

The experiment was repeated in 1937. The plots were cultivated May 8 and May 17, and sodium chlorate was first applied June 7, when the first root samples were taken. The data are given in tables 6 and 7. In both experiments a light irrigation, which served to carry the sodium chlorate into the soil, was made at regular intervals.

TABLE 6.—*Effect of combined treatment with sodium chlorate and cultivation on the carbohydrate content (percent) of whiteweed roots, 1936*

[The two sets of data for each sampling date are for the first- and second-foot levels, respectively]

SAMPLED AT TIME OF TREATMENT

Date of sampling	Undisturbed plants					Cultivated plants				
	Total sugars	Starch	Acid-hydro-lyzable sub-stances	Readily available carbohy-drates on the basis of —		Total sugars	Starch	Acid-hydro-lyzable sub-stances	Readily available carbohy-drates on the basis of—	
				Fresh weight	Dry weight				Fresh weight	Dry weight
June 1	2.24	9.67	5.15	11.91	47.83	1.61	1.94	2.82	3.55	31.42
	2.80	9.43	3.16	12.23	66.05	2.65	2.04	2.27	5.29	55.00
June 30	2.38	9.98	4.44	12.36	44.39	1.98	3.93	3.37	5.91	40.08
Aug. 2	2.77	21.24	4.74	24.01	68.57	1.95	13.65	4.59	15.60	54.54
	2.88	22.43	4.33	25.31	71.70	2.17	22.17	3.20	24.34	89.67
Sept. 1	1.98	17.04	4.86	19.02	54.81					
	2.96	20.19	3.18	23.15	76.55					
Sept 17	2.16	15.38	5.05	17.54	55.15					
	2.24	21.27	3.13	23.51	79.93					
Sept. 30	2.51	14.77	4.86	17.28	64.14					
	2.04	21.14	3.09	23.18	82.78					

SAMPLED 1 MONTH AFTER TREATMENT

Date of sampling	Total sugars	Starch	Acid-hydro-lyzable sub-stances	Fresh weight	Dry weight	Total sugars	Starch	Acid-hydro-lyzable sub-stances	Fresh weight	Dry weight
June 30	1.50	9.61	5.25	11.11	48.05	1.27	2.89	2.95	4.16	34.26
Aug. 1	2.22	11.28	4.61	13.50	49.55	1.66	6.19	3.25	7.85	48.30
	2.83	12.42	3.48	15.25	66.26	1.92	5.95	2.57	7.87	57.09
Sept. 1	2.07	14.32	....	16.39	49.30	2.13	11.55	3.90	13.68	53.85
	2.53	21.05	3.68	23.58	77.05	1.91	10.71	2.01	12.62	65.11
Sept. 30	2.34	13.20	4.83	15.54	57.98					
	2.12	20.87	3.46	22.99	85.11					
Oct. 16	2.98	11.38	3.23	14.36	52.79					
	2.77	15.55	3.64	18.32	70.46					
Oct. 29	5.20	12.83	4.99	18.03	68.04					
	4.16	18.80	3.58	22.96	88.65					

TABLE 7.—*Effect of combined treatment with sodium chlorate and cultivation on the carbohydrate content (percent) of whiteweed roots, 1937*

[The two sets of data for each sampling date are for the first- and second-foot levels, respectively]

SAMPLED AT TIME OF TREATMENT

Date of sampling	Undisturbed plants					Cultivated plants				
	Total sugars	Starch	Acid-hydro-lyzable sub-stances	Readily available carbohydrates on the basis of—		Total sugars	Starch	Acid-hydro-lyzable sub-stances	Readily available carbohydrates on the basis of—	
				Fresh weight	Dry weight				Fresh weight	Dry weight
June 7						1. 53	9. 04	2. 97	10. 57	52 53
						2. 48	13. 96	2. 46	16. 44	83. 53
July 6	2. 13	16 08	2. 62	18. 21	50. 63	2. 05	8. 70	1. 89	10. 75	41. 02
	3. 07	22. 33	1. 90	25. 40	71. 10	2. 31	5. 36	1. 66	7. 67	39 56
Sept. 13	1 81	18. 69	5. 27	20 50	68. 19					
	2 22	16. 95	11. 68	19. 17	62. 01					

SAMPLED 1 MONTH AFTER TREATMENT

July 6						3. 27	2. 75	3. 60	6. 02	30. 08
						1. 68	3. 65	1. 41	5. 33	33. 58
Aug. 9	1. 14	17. 90	2. 65	19. 04	54. 58	. 81	9. 00	4. 62	9. 81	38. 85
	1. 57	21 10	5. 79	22. 67	62. 72	1 10	8. 97	3. 34	10 07	53. 60
Oct. 12	2. 56	10. 99	1. 03	13. 55	45. 77					
	2. 91	15 93	2. 87	18 84	65. 96					

Although the data would have been more complete had samples been taken from all previously sampled plots at each sampling date,
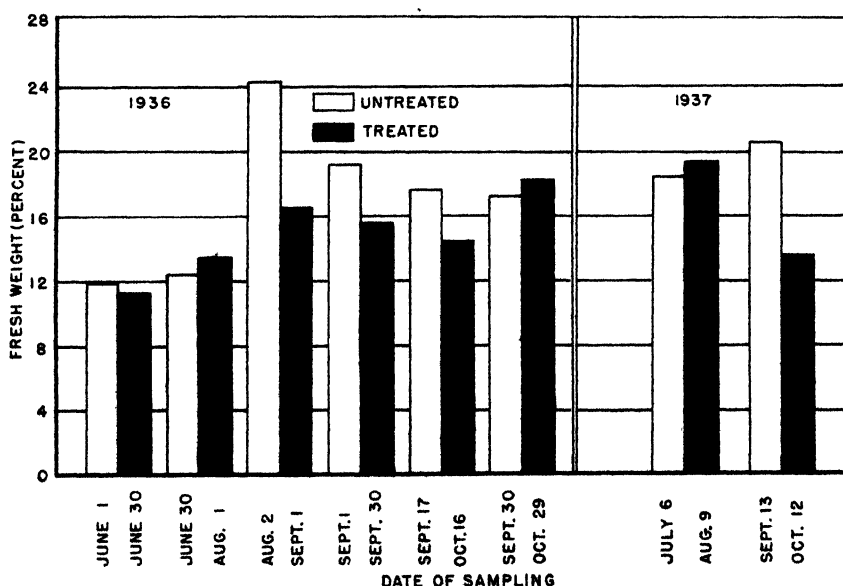
FIGURE 7.—Carbohydrate content of whiteweed roots as affected by date of application of sodium chlorate to undisturbed plots.

some interesting information is revealed by the results. The undisturbed plants showed the expected accumulation of carbohydrates.

Application of sodium chlorate to undisturbed plants had only a moderate effect in reducing the carbohydrate content in a period of 1 month after treatment regardless of the date.

A study of tables 6 and 7 and a comparison of figures 7 and 8 show that an early-season cultivation did not increase the effectiveness of the sodium chlorate applied immediately after the cultivation, which is in accord with results previously obtained with bindweed under Colorado conditions (4). On the basis of results obtained in this experiment, therefore, it is concluded that no greater reduction of the carbohydrates in the roots of whiteweed could be expected by the
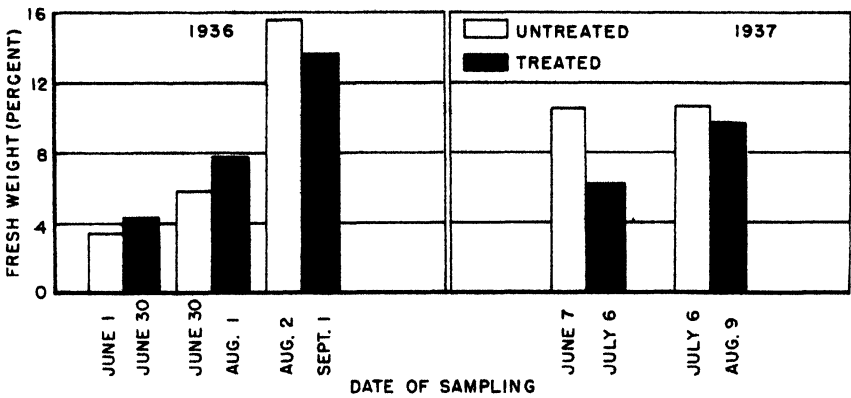
FIGURE 8.--Carbohydrate content of whiteweed roots as affected by date of application of sodium chlorate to cultivated plants.

application of sodium chlorate to cultivated plants than to undisturbed plants.

EFFECT OF FREQUENCY OF CULTIVATION ON ROOT RESERVES

The carbohydrate content of undisturbed whiteweed roots is compared in table 8 with that of plants cultivated at weekly and biweekly intervals during one season. The samples were collected September 30, 1936. Regrowth of plants on all the cultivated plots was extremely limited by the middle of July. Biweekly cultivations seemed to control the plant as well as the weekly cultivations.

The data of table 8 show very clearly the relative condition of the cultivated and undisturbed plants at the end of the growing season.

TABLE 8.—*Effect of frequency of cultivation on root reserves in whiteweed*

[Sampled September 30, 1936]

Treatment	Sugar	Starch	Acid-by-drolyzable substances	Readily available carbohydrates on basis of—	
				Fresh weight	Dry weight
	Percent	*Percent*	*Percent*	*Percent*	*Percent*
Undisturbed	2.51	14.77	4.86	17.28	64.17
Cultivated biweekly	2.41	3.43	1.56	5.84	62.14
Cultivated weekly	2.02	3.84	1.99	5.86	52.14

In these experiments cultivation was 96 to 98 percent effective in controlling whiteweed. The readily available reserve food in the remaining 4 to 2 percent of the plants amounted to about 5.85 percent. That moderately high value probably is misleading, however, a fact that becomes apparent only when it is considered that the samples upon which the analyses were made were collected from many scattered plants surving on the cultivated plots. All other samples were from areas chosen at random.

SUMMARY

The root reserves in whiteweed, *Cardaria draba* var. *repens*, have been studied. Data have been presented to show the seasonal trend of carbohydrates in both noncultivated and cultivated plots. Samples collected at 2-week intervals from April 25 to October 29 from noncultivated plots showed a maximum accumulation of carbohydrates about August 1. The lowest carbohydrate content was found early in the season when sampling was started. The total sugar content was higher in the roots from the second foot of soil than in those from the first-foot level until about the middle of September. The root reserves were present chiefly in the form of starch. The starch content of undisturbed plants reached a maximum of over 21 percent fresh weight but the total sugar content never exceeded 7.44 percent and reducing sugars remained uniformly low, reaching a maximum of 1.41 percent.

Clean cultivation held the starch content to about 4.50 percent and total sugars to less than 3.50 percent.

Both soluble and colloidal nitrogen decreased during the vegetative growth of the plants and showed a slight but apparent increase after flowering.

Samples collected at 4-day intervals for a period of 24 days after a single cultivation showed no material change in carbohydrate reserves until about 14 to 16 days after cultivation. After that there was an appreciable accumulation of carbohydrates.

Treatment with sodium chlorate accompanied by an early season cultivation did not increase the effectiveness of the chemical treatment in the control of whiteweed.

Biweekly cultivations were equally as effective as weekly cultivations in reducing the root reserves and in controlling the weed.

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